PB80-211881

Study of the Effect of Whole Animal Exposure to Acid Mists and Particulates on the Pulmonary Metabolism of Benzo(a)pyrene in the Isolated Perfused Lung Model

Cincinnati Univ., OH

Prepared for

Health Effects Research Lab.,
Research Triangle Park, NC

Jul 80

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

EPA-600/1-80-029
July 1980

PB80-211881

Research and Development

EPA

Study of the Effect of Whole Animal Exposure to Acid Mists & Particulates on the Pulmonary Metabolism of Benzo(a)pyrene in the Isolated Perfused Lung Model

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TECHNICAL REPORT DATA

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1. REPORT NO. EPA-600/1-80-029	2.	3. RECIPIENT'S ACCESSION NO. PB80 211881
4. TITLE AND SUBTITLE Study of the Effect of Whole Animal Exposure to Acid Mists & Particulates on the Pulmonary Metabolism of Benzo(a)pyrene in the Isolated Perfused Lung Model.		5. REPORT DATE July 1980 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Warshawsky, D, Niemeier, R.W., and Bingham, U.		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Cincinnati College of Medicine Department of Environmental Health 3223 Eden Avenue Cincinnati, Ohio 45267		10. PROGRAM ELEMENT NO. 1AA817 11. CONTRACT/GRANT NO. Contract No 68-02-1678
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory RTP,NC U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

Project Officer: Stephen Nesnow

16. ABSTRACT

Lung cancer represents the highest single cause of cancer deaths in the U.S. Epidemiological and experimental evidence indicates that the interplay of multiple environmental factors is responsible for the induction of lung cancer. Man is exposed to a complex mixture of potentially hazardous materials, including specific carcinogens and a variety of agents which may modify the manner in which the lung disposes of inhaled materials. One such carcinogen is benzo(a)pyrene (BaP) a ubiquitous environmental pollutant formed during the destructive distillation of coal and in other processes that involve incomplete combustion of organic material.

BaP in combustion with various agents, such as ferric oxide, has been used in animals to experimentally induce tumors of bronchogenic origin. Evidence describes the necessity for this compound, BaP, to be metabolized to produce the carcinogenic response. However, the metabolism of BaP in the lung has not been fully investigated

Since at least three enzymes are involved in the metabolism of this compound and some of these systems can be inhibited by the presence of Fe_2O_3, SO_2, or CAP to produce different metabolic patterns, a study of all the metabolites in the lung is necessary in order to determine if the rate or pattern of formation has changed. Therefore, an isolated perfused rabbit lung preparation suitable for metabolic studies has been developed to study BaP in crude air particulate and/or SO_2.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Lung cancer Benzo(a)pyrene (BaP) Environmental pollutants Metabolic patterns		06 F,T

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO OF PAGES
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/1-80-029
July 1980

STUDY OF THE EFFECT OF WHOLE ANIMAL EXPOSURE TO ACID MISTS AND PARTICULATES ON THE PULMONARY METABOLISM OF BENZO(A)PYRENE IN THE ISOLATED PERFUSED LUNG MODEL

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Contract No.: 68-02-1678
Final Report

Project Officer: Stephen Nesnow

Prepared for: Carcinogenesis and Metabolism Branch
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

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FOREWARD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

In this report, the metabolism of benzo(a)pyrene (BaP), an ubiquitous environmental pollutant and proven carcinogen, in the lung is examined. Because BaP must be metabolized to produce the carcinogenic response, an understanding of its metabolism, and the potential inhabition of that metabolism by other environmental pollutants (Fe_2O_3,SO_2,CAP), is important to the protection of human populations.

F. Gorden Hueter
Director
Health Effects Research Laboratory

PREFACE

Our general interest in the lung originated from the fact that the respiratory tract is the main portal of entry and one of the first surfaces contacted by airborne contaminants. The main reason for our interest in pulmonary disposition of pollutants is the potential importance of the ultimate toxicity of some of these agents. Of interest, also, are the agents, drugs, or pollutants reaching the lung via the circulatory system. It has been well established that the lungs are capable of binding and/or metabolizing several such agents.

There is no way to study the pulmonary metabolic activity *in vivo* because of the influence of other organs. *In vitro* tissue preparations such as slices and homogenates compromise the integrity of an investigation, especially when considering concurrent administration of multiple agents in different physical forms or when determining distribution or binding of compounds throughout the pulmonary system. Therefore, the obvious choice in our opinion was the isolated perfused lung (IPL).

A number of criteria were chosen and considered mandatory in order to provide an isolated perfused lung preparation that was sufficiently stable to permit evaluation of metabolic activity, distribution, and uptake of compounds. In addition, we thought that monitoring of physiological and biological indices would better define the stability of the system.

The system which was developed in this laboratory is relatively simple to set up and not prohibitive in cost. The major features include:
1) undiluted heparinized autologous whole blood as the perfusate;
2) recirculation of the perfusate and thus accumulation of metabolites;
3) constant pressure perfusion, perfusate level being maintained in an upper reservoir by electronic sensors; 4) chemically inert surfaces (siliconized glass or silicone rubber tubing); 5) ventilation via cycling subatmospheric pressure; 6) warming and humidification of ventilating gas (air with CO_2); 7) regulation of blood pH through infusion of sodium bicarbonate and adjustment of inhaled CO_2; 8) periodic monitoring of biochemical and physiological conditions, including blood flow, respiratory minute volume, blood gases, glucose uptake; lactate production, etc; 9) readily available sampling ports; 10) water-jacketed components; and 11) recovery of blood, lung washings, ventilating gas, trachea-bronchi, and the remainder of respiratory tract.

ABSTRACT

Lung cancer represents the highest single cause of cancer deaths in the U.S. Epidemiological and experimental evidence indicates that the interplay of multiple environmental factors is responsible for the induction of lung cancer. Man is exposed to a complex mixture of potentially hazardous materials, including specific carcinogens and a variety of agents which may modify the manner in which the lung disposes of inhaled materials. One such carcinogen is benzo(a)pyrene (BaP) a ubiquitous environmental pollutant formed during the destructive distillation of coal and in other processes that involve incomplete combustion of organic materials.

BaP in combustion with various agents, such as ferric oxide, has been used in animals to experimentally induce tumors of bronchogenic origin. Evidence describes the necessity for this compound, BaP, to be metabolized to produce the carcinogenic response. However, the metabolism of BaP in the lung has not been fully investigated.

Since at least three enzymes are involved in the metabolism of this compound and some of these systems can be inhibited by the presence of Fe_2O_3, SO_2, or CAP to produce different metabolic patterns, a study of all the metabolites in the lung is necessary in order to determine if the rate or pattern of formation has changed. Therefore, an isolated perfused rabbit lung preparation suitable for metabolic studies has been developed in our laboratory to study rate and pattern of formation of BaP in the presence of crude air particulate and/or SO_2.

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ABBREVIATIONS AND SYMBOLS

BaP	-- Benzo(a)pyrene
CAP	-- Crude Air Particulate
HPLC	-- High Performance Liquid Chromatography
ID	-- Inside Diameter
IP	-- Intraperitioneal Administration
IPL	-- Isolated Perfused Lung
IT	-- Intratracheal Administration
IU	-- International Units
3MC	-- 3-Methyl Cholanthrene
OD	-- Outside Diameter
ODS	-- Octadecylsilane
PAH	-- Polycyclic Aromatic Hydrocarbon
Pheno.	-- Phenobarbitol (PB)
POPOP	-- P-bis(2-(5-diphenyloxazole)benzene)
PPO	-- 2,5-diphenyloxazole
tlc	-- Thin Layer Chromatography

ACKNOWLEDGMENT

The authors wish to express their gratitude to Carol Warren, Connie Bools, Janet Dickman, Connie Menefee, Bernadette Nagel, and D. Gary Hancock for their expert work; Dave Yeager for metal and size analysis of CAP; Dr. Martha Radike and William Barkley for helpful discussions; and Diane Dotson for typing the manuscript.

A. INTRODUCTION

In the United States lung cancer represents the highest single cause of cancer deaths (41,58). Thus, there is an imperative need for extensive studies of causative agents, conditioning factors, and pathogenic mechanisms responsible for the development of this type of cancer. Animal models have not been developed sufficiently for the experimental study of this disease. Although many findings of carcinogenesis studies in other organs and tissues can be applied to the respiratory tract, a whole range of factors peculiar to the functional, morphological and biochemical characteristics of the respiratory organs requires a specialized study of carcinogenic mechanisms in the lung. Only recently has the potential of the lung to metabolize foreign substances been recognized as a possible factor of importance in determining the response of the lung to environmental insults (7,14,16,43-45, 50,75).

Inhalation has been the main mode of exposure in man to agents known to be casually associated with an increased incidence of respiratory cancer (41). Epidemiological and experimental evidence (8,12,23,30,32,46) indicates that the interplay of multiple environmental factors is responsible for the induction of lung cancer. Man is exposed to a complex mixture of potentially hazardous materials, including specific carcinogens and a variety of agents which may modify the manner in which the lung disposes of inhaled materials (40). It is well established that the lungs are capable of binding and metabolizing such agents (2,7,11,36,43,45,47,52,59,65,73,6,31, 66). One such carcinogen is benzo(a)pyrene (BaP) a ubiquitous environmental pollutant (3) formed during the destructive distillation of coal and in other processes that involve incomplete combustion of organic materials (6,31,66). BaP occurs as both a common contaminant of the urban environment and a constituent of tobacco smoke. In addition, its metabolites exhibit varying degrees of mutagenicity, carcinogenicity, and toxicity (24,33,70,72).

A major requirement for understanding the mechanism of BaP carcinogenesis is a detailed knowledge of the rate and pattern of formation of metabolites and the factors controlling their formation. Such factors include particulate matter, which carries a multitude of chemicals including BaP and a variety of gases may be deposited in various regions of the respiratory tract; pollutant gases and vapors may reach the deep lung with each inspiration, and particulate matter may be largely deposited in the upper and middle regions of the respiratory tract. Only the smaller particles of a few microns or less in size reach the deep lung. However, ambient air of both occupational and urban settings contains many such small particles.

It has been established experimentally that BaP in combination with ferric oxide (53) produces tumors of bronchogenic origin with an incidence of up to 100%. Carbon particles with BaP also produce a high incidence of lung tumors (57). The particulate effect has been suggested as a means of providing longer residence times at the target tissue (12,20,21,29,56,61), but the biochemical effect has not been fully investigated.

It has been shown experimentally that BaP in combination with SO_2 produced squamous cell carcinomas in the rat lung (32,67). However, based on just this one study and the fact that the control group was small, it is difficult to draw any firm conclusions about the cocarcinogenic effects of SO_2. In another study, rats exposed to high levels of SO_2 did not produce an increase in aryl hydrocarbon hydroxylase (AHH) activity of rat lung microsomes, while exposure to SO_2 followed by 3-methyl chloanthrene treatment, caused an inhibition in the AHH activity when compared to appropriate controls (26). Besides the aforementioned studies, information on the interaction between carcinogenic PAH, such as BaP, and pollutant gases, such as SO_2, is still generally lacking. Even though it is well recognized that 95-99% of SO_2 is adsorbed by the upper respiratory tract (1,42), some unknown small percentage of SO_2 will inevitably get into the lower respiratory tract. Therefore, this study attempts to determine the effects that SO_2 and particulates will have on the metabolism of BaP in the lung at levels of 1-2 ppm SO_2. This is well below the industrial threshold limit value (TLV) of 5 ppm and at levels well within exposure possibilities.

Chronic bronchitis and bronchogenic carcinoma are serious problems that affect the midregions of the respiratory tract. These diseases are probably the result of deposition of inhaled materials, such as BaP and particulates and/or SO_2, and the effects exerted by clearance of debris from the deep lung on the ciliary mucous escalator. This debris would include the inhaled particles, BaP, the metabolites of BaP, and cellular breakdown products arising from cellular injury and normal turnover. Included in these cells are the pulmonary alveolar macrophages with their engulfed particles, enzymes, and carcinogens. Of interest also are such pollutants reaching the lung via the circulatory system.

Pulmonary disposition of these pollutants has potential importance in the ultimate toxicity of some agents. While it is true that liver does the bulk of the metabolic work, it may be that the smaller fraction of the compound metabolized by or bound to the lung is responsible for disease. Inhaled carcinogens are an obvious case in point. Although the lungs receive high exposures of many inhaled contaminants, it must be emphasized that the lungs are perfused by the entire cardiac output with its supply of compounds adsorbed from the gut and perhaps those chemicals not yet removed by the liver. Agents adsorbed via the lymphatics also empty into the venous return perfusing the lungs. Thus it becomes clear that a better definition of the pulmonary capacity to metabolize and bind chemicals is necessary.

There is, however, no way to study the pulmonary metabolism of BaP *in vivo* because of the metabolic influence of other organs. *In vitro* tissue preparations, such as slices and homogenates, are not satisfactory for studies involving concurrent administration of multiple agents in different physical forms (51) or in distribution determinations or binding of compounds throughout the pulmonary system. Therefore, the isolated perfused lung (IPL) appears to be the best *in vivo* preparation for investigating pulmonary metabolism of foreign compounds (7,43-45), especially compounds adsorbed onto particulate. A number of criteria were chosen and considered mandatory in order to provide an isolated perfused lung preparation that was sufficiently stable to permit evaluation of metabolic activity, distribution, and uptake of compounds (44). In addition, monitoring of physiological and biological indices would better define the stability of the system. A summary of the major features of the isolated perfused rabbit lung preparation is presented in Table 1.

An important aspect of the current work is the assessment of the rate of formation and types of metabolites formed when BaP is administered with CAP or SO_2 on the IPL. It is well characterized at present, that the metabolic pathway progresses in three directions after possible epoxide intermediate formation: (a) isomerization and/or hydroxylation, (b) hydration of epoxides, and (c) conjugation of epoxides (4,18,28,37,48,71,74).

TABLE 1.

MAJOR FEATURES OF ISOLATED PERFUSED LUNG PREPARATION

1. Perfusate: recirculation of undiluted, heparinized, autologous whole blood
2. Constant blood pressure and blood flow
3. Chemically and biochemically inert
4. Normothermic conditions
5. Ventilation
    a. sub-atmospheric pressure
    b. net sub-atmospheric and respiratory pressure
    c. sighing
    d. fresh filtered gas - humidified and warmed
6. Monitoring of biochemical and physiological conditions
7. Controlled pH
8. Materials available for analyses
    a. blood
    b. lung washings
    c. pulmonary tissues
    d. ventilating gases

B. CONCLUSIONS

1. Enzyme Inducers

The data for various inducers on the rate and pattern of metabolism of BaP are described in Results. In general, it was found that the metabolites of benzo(a)pyrene formed by this preparation and appearing in the perfusate resemble in many respects metabolite patterns produced in the liver. Pretreatment with phenobarbital does not result in an increased rate of metabolism. Pretreatment with either 3-methylcholanthrene or benzo(a)pyrene, however, significantly increases the rate of metabolism, from 256 ng/hr/g lung to 836 ng/hr/g lung and 1290 ng/hr/g lung respectively. One of the interesting aspects of the pattern of metabolites formed is the increase in the 9,10 dihydrodiol.

A summary of the conclusions are listed below:

a. PB pretreatment interperioneally causes a decrease in BaP in the isolated perfused rabbit lung. PB may cause an increase in an alternate enzymatic pathway or this increase may simply reflect a relative increase in oxidation products as reflected by the dione formation.

b. Corn oil significantly increases total metabolism of BaP in the lung but shunts the metabolism from monohydroxylation to 7,8-dihydrodiol formation with activation of epoxide hydrase system.

c. Both BaP ip and IT pretreatment increase the metabolic rate of BaP administered IT to the IPL preparation. A change of the relative percentages of the metabolites, especially the 9,10-dihydrodiol, is evident in both pretreatment groups compared to the control. The BaP_{IP} pretreatment increased rate versus BaP_{IT} can be accounted by the corn oil administration.

d. The total metabolic rate of the 3MC group can partially be accounted for by the corn oil administration. Both IP and IT BaP pretreatment and 3MC stimulate 9,10-dihydrodiol production, whereas the corn oil increases the 7,8-dihydrodiol and the nonextractable metabolites. This interaction may also represent a synergistic effect of the PAHs with corn oil in the induction of a further epoxidation step beyond the 7,8-dihydrodiol metabolite forming the 7,8-dihydrodiol -9,10-epoxide, which has been indicated as being the ultimate carcinogen. Since this metabolite is highly reactive, the increase in the 9,10-dihydrodiol may actually reflect an increase in a further hydration product, the 7,8,9,10-tetrahydrotetrol. The latter product is indistinguishable from the 9,10-dihydrodiol on our high pressure liquid chromatographic procedure.

2. Effects of Particulate

The effects of CAP on the metabolism of BaP in the IPL are described in Results. Work from this laboratory provides evidence that chemical and physical characteristics of particles may influence in some manner the induction of tumors besides lengthening the residence time of the carcinogen benzo(a)pyrene. A comparison of the rate of formation and type of metabolites induced after various particle administration may be seen in Results.

As you will note, CAP pretreatment appears to increase enzyme activity as indicated by the rate of metabolism. CAP, however causes a decrease in the rate of metabolism that can be attributed to an increase in rate of action of macrophages or to a slow physical release of BaP from particulate. The distribution indicates that CAP pretreatment decreases the amount of nonextractable or polar metabolite and increases the 6,8- and 9,10-diol formation versus the control. This indicates that the epoxide hydrase activity causes an increase in the 9,10-diol and an increase in the diones and monohydroxylated compounds while CAP with BaP on the IPL following CAP pretreatment causes an increase in 7,8-diol formation. These data indicate that CAP on the IPL causes an increase in the epoxide hydrase activity and a slight decrease in hydroxylation and/or isomerization.

When both CAP and BaP are given together as a pretreatment followed by BaP on the IPL the rate of metabolism increases to 1093 ng/hr/g lung while the pattern is similar to CAP pretreatment alone. The rate of metabolism is not an additive effect of the CAP and BaP (830 and 1290 ng/hr/g lung).

The conclusions reached so far are described below:

1) CAP appears to act through a biological mechanism such that CAP has a cocarcinogenic effect with benzo(a)pyrene. It acts as a physical agent in decreasing the biological availability or slow release of BaP over time when administered on the lung with BaP in comparison with pretreatment of particulate only.

2) The data suggest that CAP affects BaP metabolism by two different mechanisms: One mechanism appears to be a long-term effect due to pretreatment with

particulate; this causes an increase in the total metabolic activity. The other mechanism is a short-term effect of particulate administered to the IPL; this pretreatment causes a decrease in total metabolic activity and inhibits the effects of pretreatment.

3) This work helps to partially clarify the ideas of a number of investigators: a) Particulates used in maintaining the environment of BaP in the lung for long periods appear responsible for increased tumorigenic response due to the slow release of BaP from particulate, and b) Particulates appear to influence metabolic pathways. The indication is that both factors may be responsible, i.e. slower release of BaP from particulate as measured by appearance of metabolites in blood by IPL and a significant change in metabolic pathway.

4) With concomitant administration of particulate and BaP and, therefore, a slower release rate, BaP is effectively being administered to the lung tissue in small doses when compared with BaP by itself. This type of treatment with particulate appears to be similar to previous observations in which a carcinogen is much more effective in producing tumorgenic response when given in small divided doses over a period of time as opposed to a single large equivalent dose. In addition, it is evident that there are differences in the metabolic pathways that seem to be affecting the formation of the diol expoxide, presently considered by many to by the ultimate carcinogen.

5) We are presently analyzing the kinetics and binding of BaP and its metabolites in the IPL to further assess the significance of these conclusions.

6) The results obtained are very similar whether CAP or Fe_2O_3 is used. Fe_2O_3 was used on the IPL under a grant from NCI, CA-1534403.

3. Effects of SO_2

Our attempt is to simulate environmental conditions. The 312 μg of BaP, 1 mg/kg of CAP and 1-2 ppm of SO_2 used in the experiments are realistic human exposure values; however, there are some limitations in our system. The short time period will show only an immediate effect; with SO_2 , CAP and BaP together, it may be necessary to run the experiment for longer periods of time, perhaps at higher concentrations of SO_2, or to use larger amounts of BaP and CAP to obtain dose-response relationships, or to pretreat with SO_2 by inhalation. In the presence of CAP, the SO_2 can be adsorbed on particulates and some of the SO_2 may be catalytically converted to bisulfates under the right conditions of temperature and humidity: in the presence of sunlight, the SO_2 can be converted photochemically. We are more interested at this point, however, in the effects of SO_2 on BaP metabolism under our stricter environmental conditions.

A summary of conclusions are listed below:

1) SO_2 increases the metabolism of BaP by the IPL, and affects the metabolic pattern slightly. It acts as a biological agent which causes biochemical changes in the lung due to irritation. More work in this area needs to be done in order to answer this question. The data indicate, however, that SO_2 can produce changes in the rate of metabolism of a well-defined carcinogen.

   The presence of SO_2 at 1-2 ppm with CAP and BaP together indicates that SO_2 is either adsorbed by the particulate, or that the BaP is not available for metabolism due either to BaP not being leached readily from the particulate, or to an increase in phagocytic action of the macrophage which may serve to decrease the amount of BaP available for metabolism. The particulates, it should be mentioned, can maintain the environment of the BaP in the lung for long periods of time, which may be responsible for increased tumorigenic responses and altered metabolic pathways.

   These studies are a first attempt to determine what type of interactions take place when SO_2 and/or CAP are added with BaP to a lung model system. This system appears to be a good model system for studying the metabolism of BaP. The kinetics, distribution and binding of BaP in the IPL will be analyzed to determine the importance of these initial studies.

C. RECOMMENDATIONS

It has been demonstrated in this laboratory that particulates, such as CAP, SO_2 and Fe_2O_3, affects the metabolism of BaP in the lung. Based on this information the following recommendations are presented:

1. Information on the dose, size and number of doses of BaP and/or particulate, particle size and detection of mutagens, i.e. possible ultimate carcinogens of BaP that are produced by the lung is needed in order to understand: a) the metabolism of BaP under a variety of conditions in the lung and b) the possible role that pulmonary alveolar macrophage and the lung tissue play in the mechanism of action of polycyclic aromatic hydrocarbons. This information can be used in clarifying the observed pulmonary carcinogenic effects produced in animal model systems exposed to mixtures of benzo(a)pyrene and particulate.

2. More information is needed on the kinetics of BaP metabolism, the binding of the metabolites to BaP, the structure of the S metabolite, and the better characterization of the non-extractable materials.

3. More information is needed on the effect of various particulates or BaP metabolism, distribution and kinetics in the IPL. The types of particulates that can be studied are as follows: Fe_2O_3, NiO, MnO, AlO and/or diesel fuel emissions. Additionally, the morphological and functional changes induced in the macrophages by BaP adsorbed onto the various particulates need to be determined.

4. There is a need to study the metabolism of BaP adsorbed on particulate in the whole animal. The material can be administered IT to the animal with an indwelling cannula. Metabolism could be studied over a period of days. This work is important in order to compare metabolism in the whole animal with that observed in the IPL.

5. At the same time metabolism studies are carried out (part 3 and 4), whole animal carcinogeneous studies need to be carried out in order to determine the biological responses induced by BaP adsorbed on particulate. It will be important to look at the effects of these materials given IT to the whole animal with and without pretreatment. We have found that pretreatment with and without particulate enhances the metabolic rate and changes the metabolic pattern. The underlying question is "Does an increase in metabolism shorten the latency for a biological response?"

6. Morphological and functional changes induced in pulmonary alveolar macrophage (PAM) by BaP adsorbed on particulate in long-term studies such as those described in part 5 need to be carried out. Changes in PAM can be monitored over time. These studies would involve looking for effects that may or may not be related to biological responses.

7. The IPL should be developed for use as a screening tool for the determination of potentially cocarcinogenic agents.

D. MATERIALS & METHODS

1. Chemicals

(7,10-^{14}C)-BaP, 21 mCi/mmole, is obtained from Amersham Chemical, Arlington Heights, Illinois, while unlabeled BaP is obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, and 3-methyl chloanthrene from Sigma, St. Louis, Missouri. These compounds are checked for purity by tlc and HPLC. If necessary, the cold BaP can be purified further by the use of neutral alumina column chromatography with benzene or toluene as the eluant followed by recrystallization in a benzene-isopropanol mixture.

Dr. Harry Gelboin of NCI supplied BaP metabolite standards, 3-hydroxy, 3,6-,6,12-, and 1,6-quinones, 9,10-dihydrodiol (9,10-diol), 7,8-dihydrodiol (7,8-diol), 9-hydroxy, 7-hydroxy; Dr. Ronald Harvey of Ben May Cancer Institute supplied the cis and trans 4,5-dihydrodiol (4,5-diol), 4,5-epoxide, and 4,5-quinone; and Drs. Tom Meehan, Ken Straub, and Joe Landolph of the Chemical Biodynamics Lab., University of California, Berkeley, provided the (±) 7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (7,8 diol-9,10-epoxide), and 6- and 7-hydroxy of BaP. The crude air particulate (supplied by EPA) from the Pittsburgh vicinity, analyzed by the analytical division of Kettering Laboratory for size and metal content, contains particulate size 79.0% ≤ 10 μm, 64.5% ≤ 6 μm, 47.5% ≤ 4 μm, and metals (ppm): Cd, 40; Cr, 542; Fe, 47,976; Mg, 6,482; Mn, 2,219; Ni, 1,417; Pb, 6,818; Zn, 5,496. The CAP has been partially characterized for polynuclear aromatic hydrocarbon (PAH) content. One gram of CAP is extracted with 15 ml chloroform after mixing for 3 hr; the chloroform extract is then concentrated and analyzed by HPLC with UV detector. The effluent is collected and further analyzed by fluorescence. The following compounds have been identified by flourescence and known standards: carbazole (43.7 ppb), fluoranthene (77.9 ppb), pyrene (66.8 ppb), benzo(k)fluoranthene (141 ppb), benzo(a)pyrene (32.8 ppb), benzo(ghi)perylene (47.8 ppb). Benzo(c)-phenanthrene and phenanthrene have been tentatively identified (Table 2, Fig. 1).

For scintillation counting, PPO (2,5-diphenyloxazole) and Scintiverse media & POPOF [(p-bis-(2-(5-phenloxazol benzene)] are obtained from Fisher Chemical, Cincinnati, Ohio, while Triton X100 is obtained from Rohm and Haas, Cincinnati, Ohio and corn oil is obtained from Mazola Best Foods, Englewood Cliffs, N.J.

Heprin is purchased from Abbott Laboratories (North Chicago, Ill.), sodium phenobarbital, lidocaine 2% and epinephrine from Parke Davis (Detroit, Michigan). Pararosaniline is obtained from Eastman Kodak (Rochester, N.Y.) and ninhydrin spray from Sigma (St. Louis, Mo.).

All solvents used are redistilled except methanol (Fisher Scientific, Fairlawn, N.J., spectroanalyzed and HPLC grade).

2. Instrumentation

All ^{14}C scintillation counting is performed on Tri-Carb Packard Liquid Scintillation Spectrometer (3200 and 2002) and fluorescence is recorded on an American Instrument Corrected Unit. IPL samples are chromatographed on a Varian 8500 HPLC with variable uv wavelength detector under the following conditions: Varian reverse phase ODS 25 cm x 2 mm columns, 10 μm particle size, methanol-water mixture from 62% to 100% methanol; increasing 1%/min x 6, 0% for 3 min, 3%/min x 3; 3%/min x 6 and 4%/min x 6; room temperature; monitor at 268 nm; flow; 1 ml/min.

3. Isolated Perfused Lung

a. Perfusion Apparatus

The system for perfusing the lung (Figure 2,3) consists of three integrated components: 1) the ventilating gas; 2) perfusing apparatus; and 3) an artificial thorax. The portion of the system in direct contact with the blood is glass with the exception of silicone rubber tubing (Size AF - New Brunswick Scientific, International) used in the peristaltic pump. The glass is treated with Siliclad according to directions of the manufacturer (Clay Adams).

1) Ventilating Gas

Filtered air is provided by a diaphragm type air pump (Figure 2,3). The rate of flow of carbon dioxide and air are measured with rotameters and the rate regulated by adjustable clamps. The gases are mixed and their entry into the system is regulated by a solenoid valve (A). This solenoid, as well as an additional one in parallel (B), are activated by the movement of a rubber breathing bag against a double-pole microswitch. The solenoids operate in unison, one opened and one closed (see Figure 5). This provides a stream of gas at a constant pressure (0.25 cm H O) to the lung.

The ventilating gas is warmed before it enters the lung by passing it through a glass coil submerged in a waterbath, maintained at 37°C, and is then humidified by bubbling through a coarse glass frit washing bottle. Excess water is removed by a trap. After passing through a rubber breathing bag, undirectional flow is maintained by a one-way valve, and the pressure is monitored by a water manometer. A three-way teflon, 4 mm bore, stopcock (G_1) permits the addition of other gases, aerosols, etc. to the ventilating gas.

FIGURE 1

Composition of Crude Air Particulate

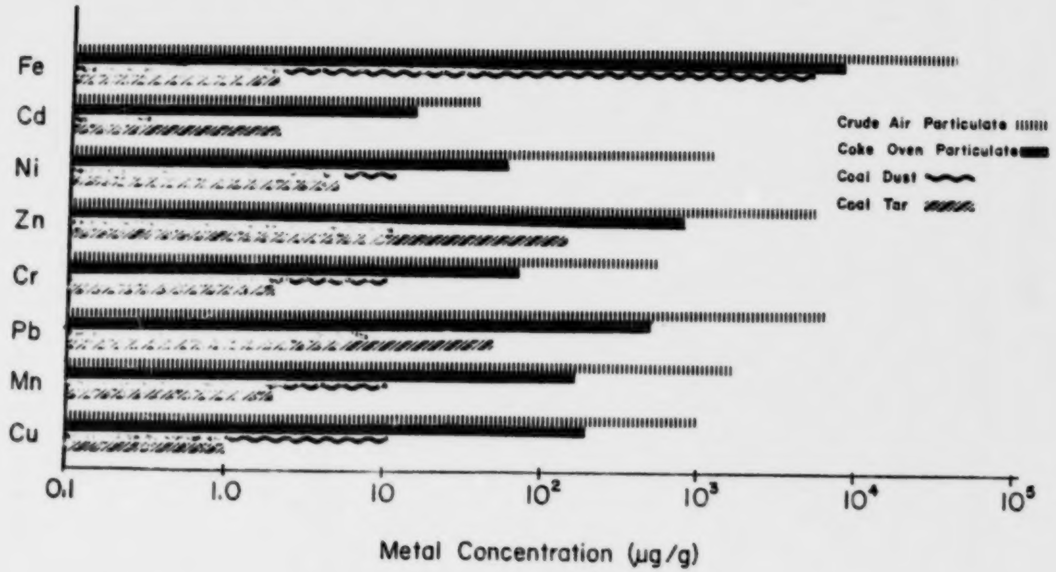


TABLE 2.

EMISSION SPECTROGRAPHIC ANALYSES OF CRUDE (AIR) PARTICULATE MATTER

Metal	Concentration (ppm)*
Aluminum	10,000
Antimony	0
Barium	1,000
Beryllium	0
Bismuth	0
Boron	500
Calcium	10,000
Cobalt	10
Copper	1,000
Molybdenum	1,000
Phosphorus	5,000
Potassium	0
Silicon	10,000
Silver	0
Strontium	500
Tellurium	0
Thallium	0
Tin	500
Vanadium	500

*These sensitivities may be too low for the more volatile metals such as antimony, thallium and tellurium.

A fork-shaped tube with one-way valves joins the ventilating gas system through a tee-connection that permits the lung to respire fresh ventilating gas with each inspiration. Extratracheal dead air space is 2.5 cc. The expired gas may be diverted to a spirometer or to an exit gas line through a three-way teflon, 4 mm bore, stopcock (G_2). (See Modification Section e)

2) Blood Perfusion System

A water jacketed blood reservoir (manufactured by Thomas Curcar, 792 Kenray Court, Cincinnati, Ohio) (50 ml) provides a constant hydrostatic pressure (23 cm H_2O measured at the hilus) to the cannulated pulmonary artery (Figure 4). The level of blood is maintained in the reservoir by a sensing device (Dyna Sense electronic liquid level controller - Cole Parmer) using platinum-tipped electrodes which control an off-on switch to a peristaltic pump (Harvard Apparatus - Model 1210). The teflon, 2 mm bore, stopcocks, (R_1 and R_2) are used only in the start-up procedure to ensure air-free lines. Blood drains freely from pulmonary vasculature and is exposed to the cycled sub-atmospheric pressure in the artificial thorax. The bottom portion of the artificial thorax serves as a reservoir for the venous blood.

The pH is monitored continuously by placing the electrode directly into the 50 ml reservoir through a port in the plexiglass top.

An infusion pump (Harvard Apparatus - Model 1131) delivers the additives, i.e., glucose and dosium bicarbonate, at a constant rate into a port of the 50 ml blood reservoir.

3) Artificial Thorax

A temperature of 37°C is maintained in the entire system by circulating the water from the constant temperature bath (37°C) through the jacketed blood reservoir and artificial thorax by means of a submersible water pump (Figure 2 - Sargent Welch Sci. Co. S-7151C). A plexiglass lid, fitted with a rubber "O" ring, and scaled with silicone high vacuum grease is held in place on the ground glass rim of the thorax.

Three ports protrude from the lid. One port is connected to the small animal respirator (Harvard Apparatus - Model 662) which is operated in reverse. Another port provides the controlled vacuum source and the third port leads to a Magnehelic gauge (Dwyer Instruments Inc.) to monitor thoracic pressure.

Four glass prongs, which extend horizontally from inside the artificial thorax, provide a rest upon which a circular piece of tygon tubing rests. Gauze strips saturated with distilled H_2O are hung from the tubing and provide a humidified interior.

FIGURE 2

IPL Design

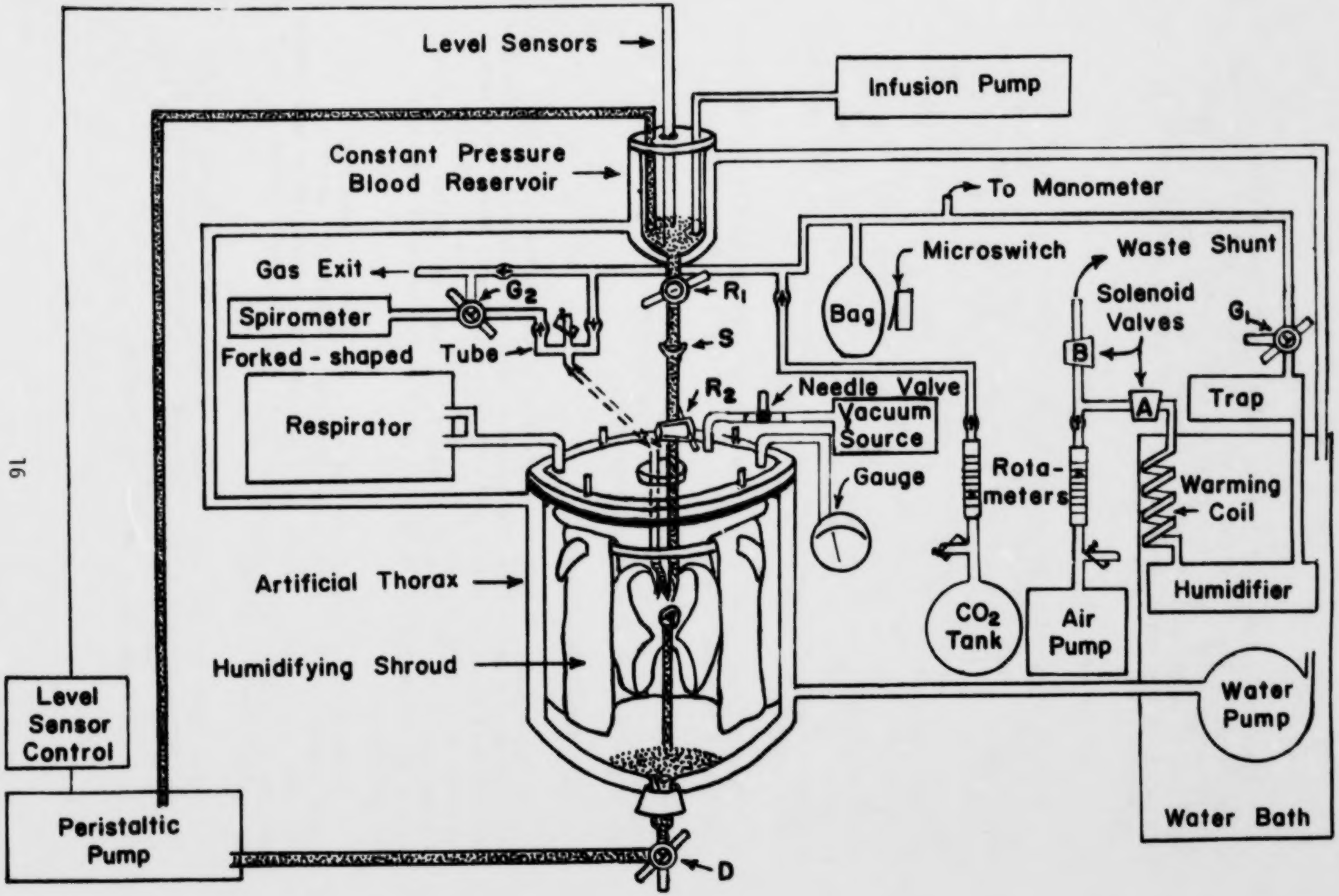

Level Sensors
Infusion Pump
Constant Pressure Blood Reservoir
To Manometer
Gas Exit
G_2
R_1
Microswitch
Waste Shunt
Spirometer
Bag
Solenoid Valves
G_1
Forked - shaped Tube
S
B
R_2
Needle Valve
A
Trap
Respirator
Vacuum Source
Gauge
Rota-meters
Warming Coil
Artificial Thorax
Humidifier
CO_2 Tank
Air Pump
Humidifying Shroud
Water Pump
Level Sensor Control
Peristaltic Pump
Water Bath
D

FIGURE 3

Simplified IPL Schematic

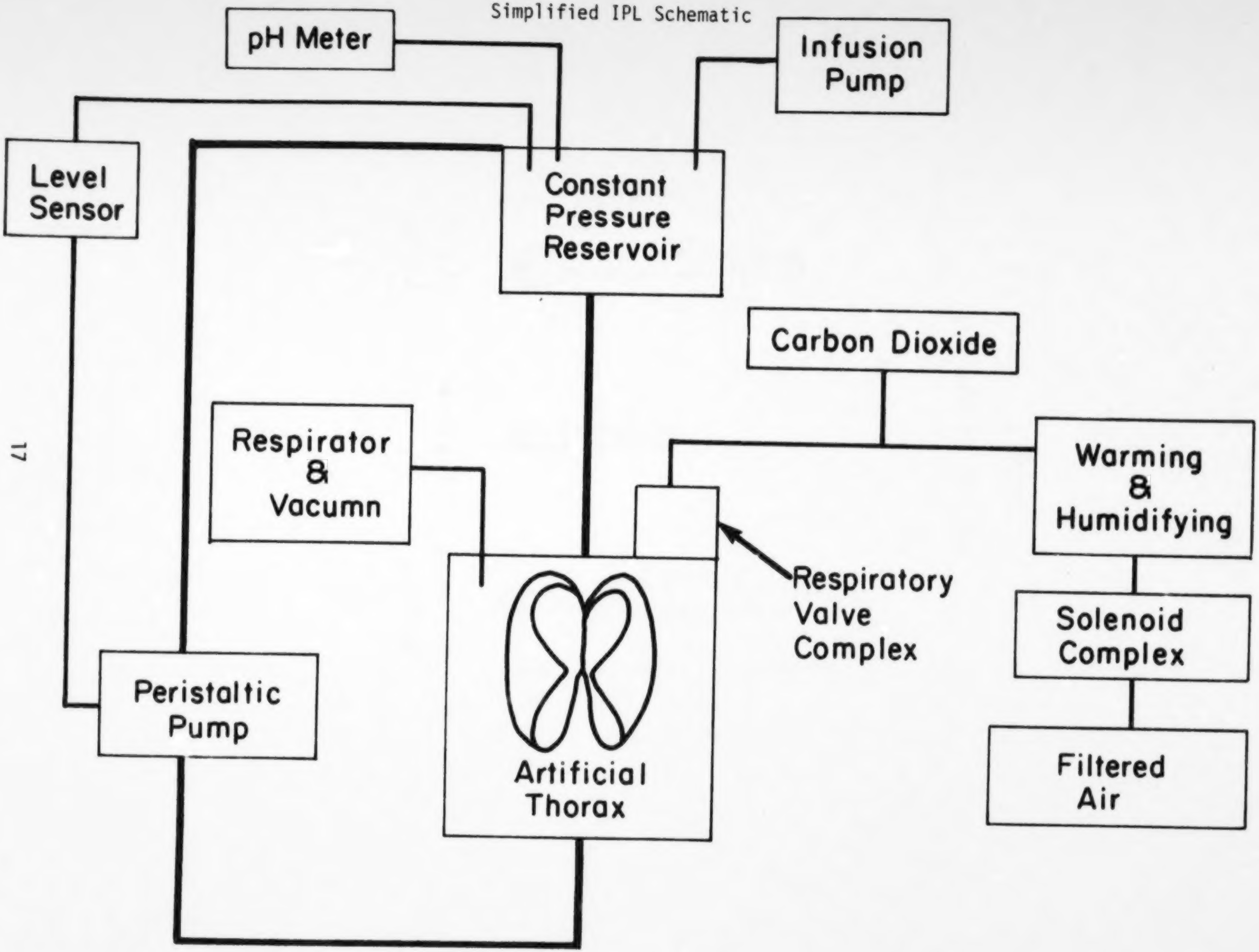

pH Meter
Infusion Pump
Level Sensor
Constant Pressure Reservoir
Carbon Dioxide
Respirator & Vacumn
Warming & Humidifying
Respiratory Valve Complex
Solenoid Complex
Peristaltic Pump
Artificial Thorax
Filtered Air

FIGURE 4

Cannulae for IPL

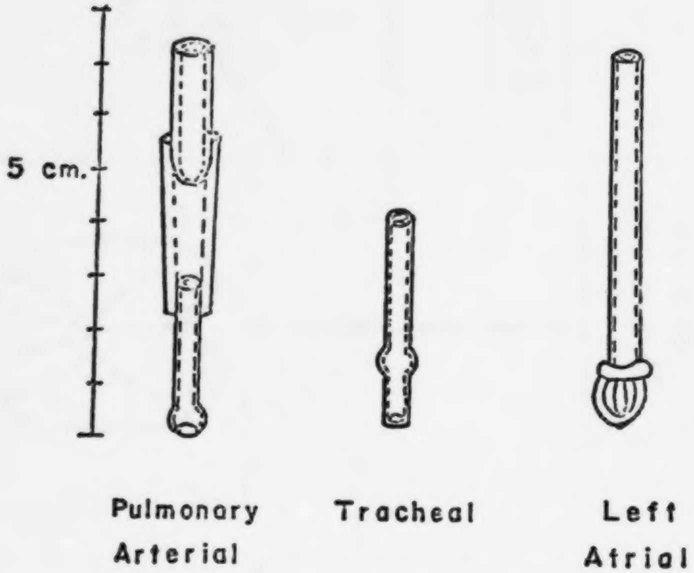
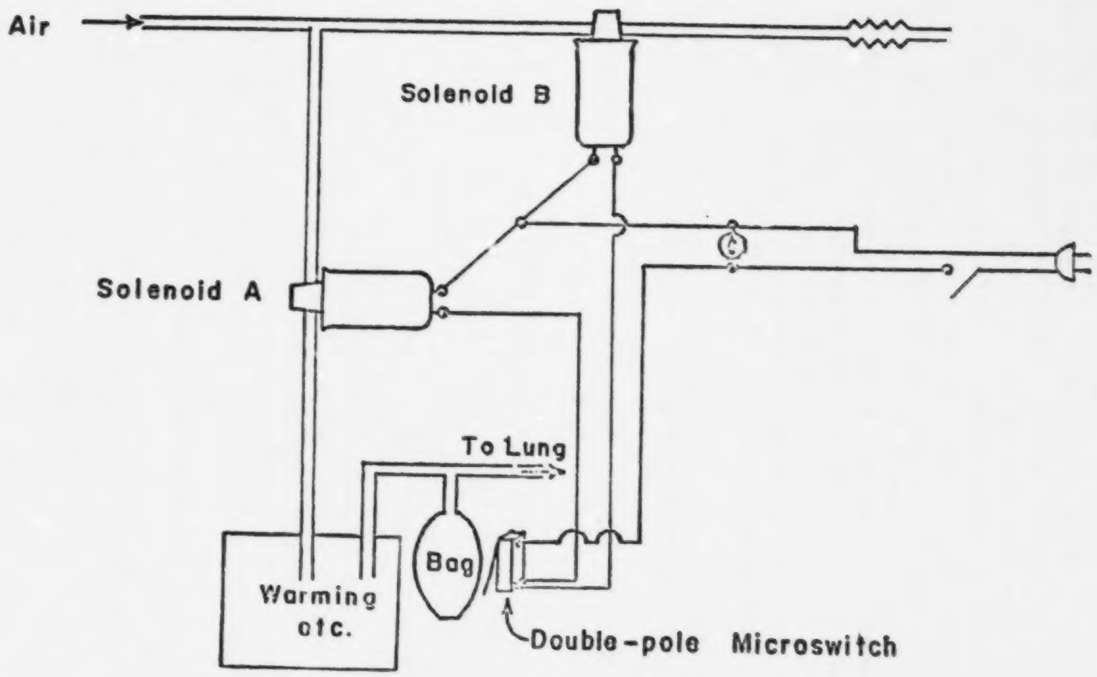

5 cm.
Pulmonary Arterial
Tracheal
Left Atrial

FIGURE 5

Solenoid Diagram


Air
Solenoid B
Solenoid A
To Lung
Bag
Warming
etc.
Double-pole Microswitch

b. Preparation of Lungs

The rabbit is tied on a specially designed slanted board with the head below the elevation of hind legs and the hair removed with electric clippers from the ventral side. Heparin (1000 IU/kg body weight) is injected intravenously into the median vein or central artery of the ear. Five minutes a cardiac puncture is made using disposable 60 ml syringes (18 gauge needle). At least 85 ml of blood is needed to prime all lines, and to sample periodically from the system. (Our experience has enabled us to figure on recovering at least 21 ml of blood per kg body weight.) Care is taken to enter between the sixth and seventh ribs next to the sternum so as not to puncture the lungs. Immediately following the cardiac puncture the rabbit is killed by a sharp blow to the head or CO_2 inhalation. A midline incision is made from the neck to the abdomen to expose the trachea and rib cage. The liver is retracted and the diaphragm cut on both sides to collapse the lungs. The heart and lungs are exposed through a midline sternotomy and the rib cage retracted. Additional blood may be recovered at this point if an adequate amount was not obtained via cardiac puncture.

The trachea is then cannulated using a siliconized (Siliclad-Clay Adams) glass tube (3 mm ID, 4 mm OD by 3.5 cm in length) and ligated (see Figure 4). The trachea, lungs and heart are dissected free from their attachments, taking care not to puncture the lungs, and kept moist with physiological saline.

The pericardium is removed and the pulmonary artery cannulated with a siliconized glass tube (3 mm ID, 4 mm OD, by 6.5 cm in length) previously filled with heparinized blood and ligated. During this procedure care must be taken not to introduce air bubbles into the vasculature or an immediate cessation of flow occurs. The entire right ventricle and right atrium, together with most of the left ventricle (up to 0.5 cm below the A-V septum) is removed. The left atrium is cannulated by passing a siliconized glass tube (3 mm ID, 4 mm OD, by 7 cm in length) through the remaining left ventricle and bicuspid valves to the atrium. The cannula is secured with a ligature and the remaining tissue dissected free. The preparation is weighed.

c. Starting the System

One hour prior to perfusion, the water bath and pump are switched on so that the temperature of the system reaches 37°C. All tubing that carries blood, the blood reservoir, and the artificial thorax are flushed with physiological saline and drained through a 3-way teflon, 2 mm bore, stopcock (D). The arterial tube is filled with heparinized blood up to the ball and socket joint, a 12/5 fitting (S), and the teflon stopcock (R_2) closed. The reservoir is filled to the proper level taking care to avoid bubbles of air from becoming trapped in the lines and then the teflon stopcock (R_1) is closed.

The lung preparation is suspended in the artificial thorax by connecting the tracheal and pulmonary arterial cannulae, using silicone rubber tubing, to the appropriate glass tubes. The tubes pass from the thorax through a No. 8 rubber stopper mounted in the plexiglass lid.

At this point the 50 ml reservoir is brought into position and the ball and socket joint clamped. Stopcocks R_1 and R_2 are opened to allow the blood to flow freely. All blood previously collected is slowly added to the reservoir and the peristaltic pump turned on, intermittently, to fill the tubing. As soon as all tubes fill, the remaining blood is added, followed by heparin, and epinephrine. The constant level probes, extending through a plexiglass top, are placed into the reservoir to ensure a constant blood pressure of 23 cm of blood measured from the hilus of the lung, and the peristaltic pump is activated. It is emphasized that extreme care must be taken so as not to introduce air bubbles into tubing carrying blood between the blood reservoir and the lung via the pulmonary artery.

The remaining connections on the lid are completed. The air pump, CO_2 cylinder, rotameters, and solenoid valves are activated or adjusted (air flow = 3.3 L/min., CO_2 5%) allowing the prewarmed and humidified gas mixture to flow.

The vacuum source is then turned on and adjusted by a needle valve to reduce the pressure inside the artificial thorax initially to -25 to -20 cm H_2O, which opens the collapsed lungs. The respirator is started and alternating sub-atmospheric pressures (-3 to -12 cm H_2O) are maintained by adjusting the stroke volume of the respirator and the needle valve. The frequency of breathing is kept at 50 respirations per minute.

At this point, the system is completely automated except for occasional large sub-atmospheric pressure excursions (-30 cm H_2O) every 15 minutes, to sigh the lungs by closing the needle valve.

Glucose (30 mg/hr) and sodium bicarbonate (0.3 mEq/hr) are added to the reservoir with a constant infusion pump at a rate of 0.3 ml/hr. The pH (7.35 to 7.45) is maintained by adjusting the quantity of CO_2 mixed into the air stream. Additional heparin and epinephrine are added as needed to the blood through the blood reservoir or administered through the forked-shaped tracheal tube if fluid, or if gas or aerosol, by the teflon stopcock (G_1). Blood samples may be drawn from either stopcock (D) or the reservoir at various intervals of time throughout the perfusion.

The rate of blood flow was estimated by allowing continuous flow through the peristaltic pump, calibrated at 37°C and measuring the volume of blood collected per unit of time. Net lung weight gain was used as one indicator of edema formation.

d. Cleaning the System

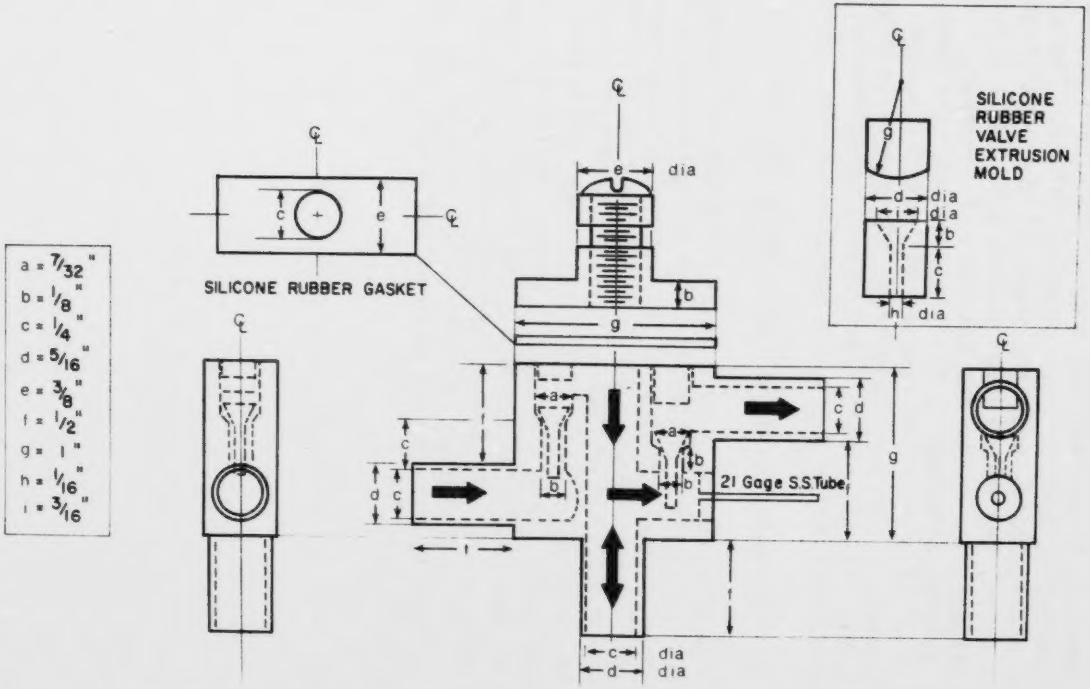
Immediately after an experiment, the system is flushed three times with distilled water. The system is then partially filled with a 10% solution of Isoterge (Scientific Products), and allowed to circulate for 15 minutes. This is then followed by five additional rinses with distilled water. The cannulae are soaked in an Isoterge solution for 2-3 hours and rinsed thoroughly with distilled water. The silicone rubber tubing is changed after each perfusion.

e. Modification

One of the aims of this study has been to study BaP coadministered intratracheally with CAP or SO . A development which arose from this need was the tracheal valve system which is shown in Figure 6. The valve is fabricated with Teflon and has an extra tracheal dead air space of approximately 6 CM . Silicone rubber stem valves permit unidirectional flow. The offset diagram in Figure 6 gives the dimensions of the valve extension mold which is also fabricated with Teflon. Intratracheal pressures can be measured and intratracheal instillations are made through a point at the top of the valve. Spirometric measurements are also possible, therefore adding another diversion to metabolic and acute toxicity investigations. This modification replaces G_2 and the spirometer indicated in Figure 2 and the tracheal valve is connected to the fork-shaped tube.

FIGURE 6

Tracheal Value Schematic


a = 7/32"
b = 1/8"
c = 1/4"
d = 5/16"
e = 3/8"
f = 1/2"
g = 1"
h = 1/16"
i = 3/16"
SILICONE RUBBER GASKET
SILICONE RUBBER VALVE EXTRUSION MOLD
21 Gage S.S.Tube
℄
dia
a
b
c
d
e
f
g
h
i

f. Pretreatment of BaP, CAP, 3MC, Phenobarbital and Corn Oil

IP pretreatment with BaP or 3MC, 20 mg/kg in corn oil vehicle is performed 24 hours before sacrifice, sodium phenobarbital 50 mg/kg in saline on three successive days with last dose 24 hours before sacrifice and corn oil, 3 ml/kg, 24 hours before sacrifice. Intratracheal injection of 10 mg/kg of CAP and/or BaP based on work by Saffiotti (53) with hamsters is carried out once a week for 5 weeks. The particulate is suspended in 2 ml of physiological saline. The animal is restrained on the rabbit board, with the level of the head slightly below the level of the heart, the neck region shaved with small animal clippers, and then palpated to locate the trachea. A 16-gauge, 1.5 inch needle with a 12-ml syringe attached is inserted through skin into the trachea. If 8 to 10 ml of air can be removed without resistance, the needle is in the trachea. Following injection, the needle is removed, the head of the rabbit raised (rabbit board tilted to 45° angle), and the animal is forced to breathe deeply by applying periodic pressure to diaphragm.

g. Administration of BaP, CAP to IPL

One microcurie of pure (7,10-^{14}C)-BaP (21 mCi/mmole, Amersham/Searle, Arlington Heights, Ill.) is diluted with unlabeled pure BaP (Aldrich Chemical Company, Milwaukee, Wis.) and evaporated gently to dryness under nitrogen. It is then taken up to a final amount of 1.24 μmoles of BaP (0.8 μCi/mmole) or 312 μg of BaP in a 1-ml ethanolic saline (1:1) solution and is intratracheally injected on the IPL. When BaP and particulate are added together on the IPL, the solutions of labeled and unlabeled BaP are slowly evaporated under a stream of nitrogen so that the BaP is adsorbed onto particulate and then taken up in 1 ml of saline and intratracheally injected on the IPL. In each case the syringe is rinsed once with an additional milliliter of saline and injected on the IPL.

h. SO_2 Administration *In Vivo* and *In Vitro*

For the generation of SO_2, a stream of dry compressed air is passed over a 2 inch 3/16 in. i.d. x 0.30 in. wall thickness FEP teflon permeation tube with FEP teflon plugs maintained at 37°C containing condensed SO_2 (54). This SO_2-air stream is then mixed with either humidified air containing 5% CO_2 from the ventilating system of the IPL before entering the IPL preparation via the tracheal valve (Fig. 7) or dry filtered room air before entering the tracheotomized rabbit (Fig.8). The exhaled and excess SO_2-air is then bubbled through two sodium hydroxide scrubbers and a dessicating column before it is vented out of the system by a house vacuum. The concentration of SO_2 is adjusted by carefully controlling the following systems: the stream of compressed air over the permeation tube; the dry room air (*in vivo*) or humidified CO_2-air (*in vitro*) and the vacuum source. A critical resistance consisting of a 3 in. piece of P.E. 60 tubing is used to measure flow rate of the compressed air. The West Gaeke method, a colorimetric determination of sulfur dioxide concentration (ppm) is used (27,69). Air is sampled for specified periods of time throughout the experiment by a midget impinger containing 10 ml of 0.04 M potassium tetrachloromercurate absorbing reagent located on the exhaust side of the system. Two ml of a 0.016% pararosaniline (Eastman Kodak Co., Rochester, N.Y.) reagent and 1 ml of a 0.2% formaldehyde solution are added to the absorbing reagent, transferred to 25 ml glass stoppered graduated cylinder, mixed, and the purple color is allowed to develop for 20 minutes at which time the absorbance is determined at 575 nm on a Beckman μv spectrophotometer. The microliters SO_2 of the samples are determined from standard calibration curves of absorbance (range 0 to 0.6) versus microliters or micrograms of SO_2. The standard curve is prepared daily. Concentration (ppm) of SO_2 is determined by dividing microliters SO_2 by the flow (liters/min) that the vacuum draws through the impinger. The entire system is equilibrated with the SO_2 in line to obtain 1.5 - 2 ppm before placing the rabbit or the lung in line and the SO_2 is then monitored for the duration of the experiment. The SO pretreatment is performed by restraining the animal on the rabbit board with the level of the head slightly lower than the level of the heart and the neck regions shaved with a small animal clipper. One cc of local anesthetic, lidocain 2% in an epinephrine solution (W. A. Butler Co., Columbus, Ohio) is administered subcutaneously to several sites, injecting a total of 3 to 4 cc. After 5 minutes, a 1 inch midline incision is performed, the trachea isolated, a small horizontal cut is made in the trachea, and the cannula inserted and ligated. A tee connection in line with the smallest possible distance to the cannula is attached to the cannula through which the rabbit breathes. An additional air reservoir is added to aid the rabbit in case of a sudden loss of positive pressure. A slight positive pressure is maintained by monitoring a respiratory bag placed in line immediately after the rabbit and before the air trap. A rotameter placed on the inhalation side of the rabbit is used to monitor the rabbit's respiration. In these experiments the average respirations prior to tracheotomy are 167 respirations/minute and after tracheotomy 153 respirations/minute while the pulse rate remains unchanged at 80 pulses/minute.

FIGURE 7

SO_2 Modification of IPL

IN VITRO

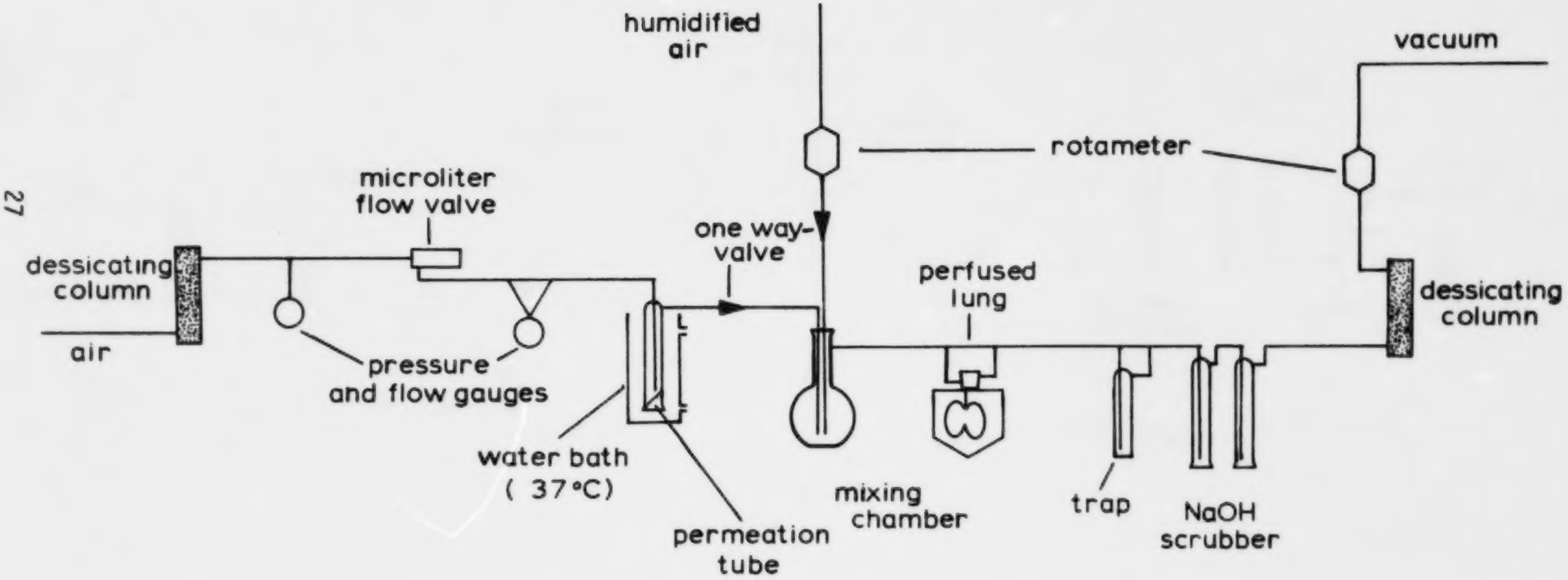
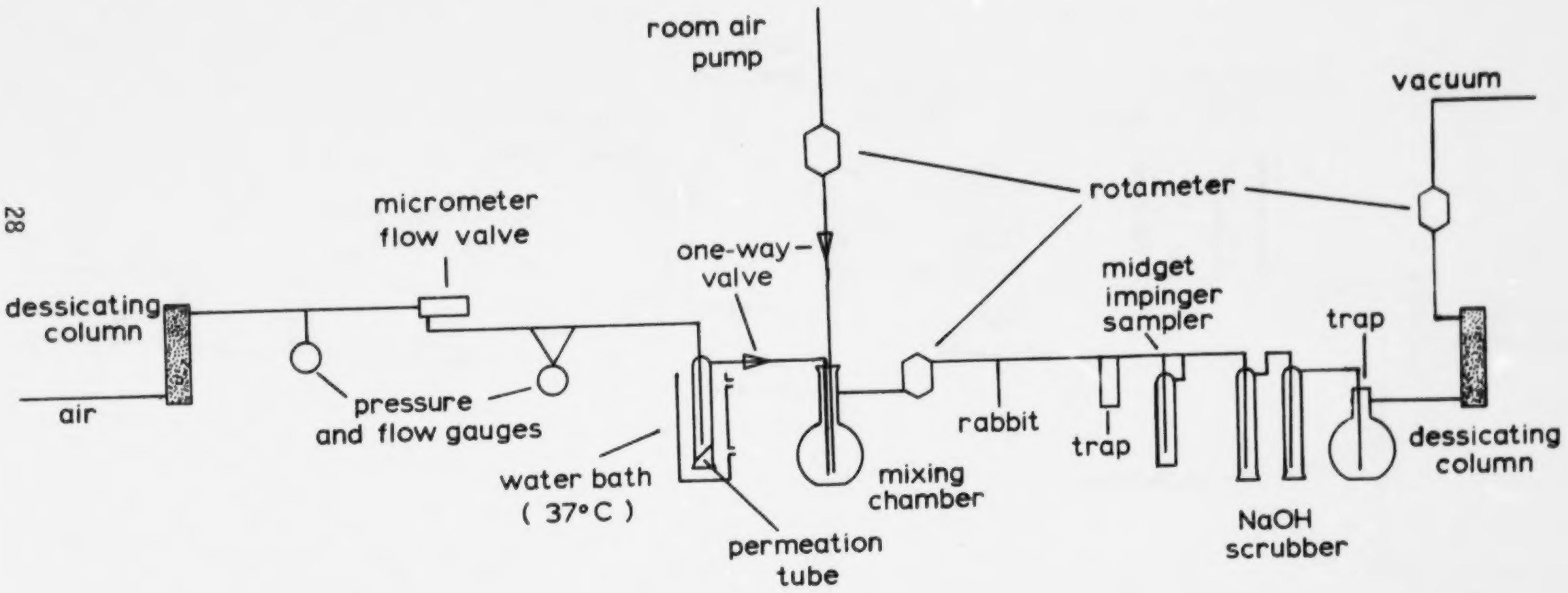

humidified air
vacuum
rotameter
microliter flow valve
one way-valve
dessicating column
perfused lung
dessicating column
air
pressure and flow gauges
water bath (37°C)
permeation tube
mixing chamber
trap
NaOH scrubber

FIGURE 8

SO_2 Modification of IPL

IN VIVO


room air
pump
vacuum
rotameter
micrometer
flow valve
one-way-
valve
midget
impinger
sampler
dessicating
column
trap
air
pressure
and flow gauges
rabbit
trap
dessicating
column
water bath
(37°C)
mixing
chamber
permeation
tube
NaOH
scrubber

i. Extraction and Analysis of Biological Samples

5.5 ml blood samples are taken from the perfusion system at 15,30,60,90,120, and 180 minute intervals. Of this volume, 0.5 ml is placed in a glass scintillation vial, digested with 0.5 ml 1N NaOH in a 60°C oven overnight, bleached with 0.5 ml tert-butyl hydroperoxide in a 60°C oven for thirty minutes, and then counted with 15 ml of Scintiverse (Fisher Scientific) Scintillation Media with internal standard. This sample is the pre-sample, i.e. prior to extraction. The remaining 5 ml of blood for each sample is extracted twice with 30 ml acetone, benzene, isoamyl alcohol (10:13:0.1) by shaking for 30 minutes and then centrifuging at 1500 rpm, 5°C, for 30 minutes. The organic phase is removed and evaporated to dryness in a 50°C water bath, under nitrogen and stored in the cold until analysis. The remaining tissue residue (post extraction) is digested in 10 ml 1N NaOH overnight in a 60°C oven and a 0.5 ml sample is bleached and counted as above. The organic portion is reconstituted in benzene (0.06 ml) for thin layer chromatography (TLC) or in chloroform (0.15 ml) for high pressure liquid chromatography (HPLC).

When the lungs have been removed from the perfusion system they are weighed (to determine weight gain due to edema) and then lavaged three times with physiological saline (5 ml/g lung tissue). This "washout" fluid is then centrifuged at 1500 rpm, 5°C for 50 minutes in 250 ml centrifuge bottles. The supernatant is decanted in a 500 ml graduated cylinder. The pellet, containing pulmonary alveolar macrophages and any possible particulate remaining from injection, is transferred to a graduated centrifuge tube along with approximately 20 ml of "washout" fluid and recentrifuged. The supernatant fractions are combined in the 500 ml graduated cylinder. This volume is recorded, and 1/3 of this volume is extracted twice in a 250 ml separatory funnel with an equal volume of extraction solvent. A pre-washout 0.5 ml sample is digested in 0.5 ml 1N NaOH in a 60°C oven overnight, bleached and counted as above samples. Following the second extraction, the tissue residue volume is measured, 0.5 ml (post sample) is digested with 0.5 ml 1N NaOH in a 60°C oven overnight, bleached, and counted. The organic portion of the washout is evaporated in a 50°C water bath, under nitrogen and stored in cold until further analysis. The organic portion is reconstituted in benzene (0.06 ml) for TLC or (.15 ml) chloroform for HPLC.

The macrophage pellet from above is resuspended in distilled water to a total volume of 5.0 ml, 0.5 ml is digested, bleached, and counted and the remaining 4.5 ml is extracted twice with 30 ml of extraction solvent, and centrifuged. The organic portion is evaporated under nitrogen in a water bath as in above sample and stored in cold until analysis. The organic portion is reconstituted in 0.6 ml benzene for TLC and .15 ml chloroform for HPLC. The tissue residue is digested with 10 ml of 1N NaOH overnight in a 60°C oven, volume recorded, and

0.5 ml bleached and counted as described above.

After the lungs have been lavaged, all extraneous tissue is removed (including left atrium of heart, fat, etc.) then the lung tissue is scraped from the trachea bronchi. This is done by scraping the tissue with a scalpel. After as much lung tissue has been removed as possible, the weight of that tissue, as well as the weight of the trachea-bronchi is determined. The lung tissue is homogenized in a Waring Blender (2 minutes) with sufficient distilled water to make a total volume of 75 ml, and a 0.5 ml sample is digested, bleached and counted. After homogenization of the lung tissue, 10 ml is placed in a 50 ml graduated centrifuge tube and extracted twice with extraction solvent. After the second extraction, the organic portion is evaporated under nitrogen in a water bath and stored in cold until analysis. The organic portion is reconstituted in 0.6 ml benzene for TLC or .15 ml chloroform for HPLC. The tissue residue is digested with 10 ml of 1N NaPH in an oven overnight, post volume recorded, and a 0.5 ml sample bleached and counted.

The trachea bronchi are cut in small pieces, placed in a 50 ml graduated centrifuge tube, and extracted twice with acetone, benzene, and isoamyl alcohol. No presample is able to be taken from the trachea bronchi. The organic portion is evaporated under nitrogen, stored in cold until analysis, and reconstituted in 0.6 ml benzene for TLC and .15 ml chloroform for HPLC. The tissue residue is digested with 10 ml of 1N NaOH overnight in a 60 C oven. The post volume is recorded, and a 0.5 ml sample is bleached and counted.

Metabolite standards are dissolved in benzene or methanol for TLC and in methanol for HPLC. A portion of the reconstituted benzene sample is chromatographed on TLC (0.1 mm silica gel plates, Eastman Kodak 6061) using benzene·ethanol (19:1). Spots are located in the μv light, the entire chromatogram is cut into strips, and the spots are quantitated by counting using a cocktail of POPOP and 4 g PPO per liter of toluene. Identification is made chromatographing metabolite standards with each experimental sample.

An HPLC chromatogram is recorded using the standard mixture (Fig. 9,10) once each morning. The chloroform sample is then chromatographed and fractions collected with time, such that each of the peaks and spaces between peaks are collected individually. The fractions are then quantitated by counting using a cocktail of toluene:Triton X100: ethanol (8:4:3) plus PPO (6 g/liter) and POPOP (25 g/liter). For both TLC and HPLC, amounts of each metabolite are determined for each sample in nanograms per gram lung wet weight used for each perfusion. The total rate of appearance of metabolites (ng/hr/g lung) in the blood is based on a linear regression of a time count study from 0 through at least 90 minutes. The metabolites in the washout, lung, trachea bronchi, and macrophage are also determined in nanograms per gram lung wet weight. The trachea bronchi is initially determined based on trachea weight and then is corrected to lung wet weight. All data collection, data reduction, and statistics are handled by computer programs. All samples are processed under nitrogen and subdued yellow lighting to minimize photo-oxidation.

This work was begun using TLC methods before HPLC methods were routinely used in the laboratory. Therefore, the HPLC data presented validate the TLC data as indicated in Results. The TLC blood data are presented as the slope over the range of 0-120 minutes and as distribution data at 180 minutes. The HPLC blood data are compared to the TLC blood data either at 60 minutes only or using the slope over the range of 0-120 minutes.

The data for the 9,10- and 4,5-diols are directly comparable between the two techniques. The monohydroxylated metabolites on TLC are composed of ^{14}C-activity under the 9- and 6-OH and 3- and 7-OH peaks on HPLC. Dione metabolites on TLC are composed of ^{14}C-activity under the 4,5-quinone, 3,6-,6,12- and 1,6-quinones and 4,5-epoxide (or its derivative) peaks on HPLC. The 7,8-diol and the S metabolite on HPLC, chromatograph together on TLC.

Scintillation counting indicates that an unknown (S) metabolite chromatographs about 1 minute after BaP. When this fraction is collected and chromatographed on TLC in benzene:ethanol (19:1) ^{14}C-activity appears in a spot above baseline which is both fluorescent and ninhydrin positive. This suggests that the S metabolite is either an extractable peptide conjugate or part of the nonextractable metabolite (S) which chromatographs very differently on HPLC and TLC. The S metabolite varies with _in vivo_ treatment and this suggests that the metabolite is an extractable peptide conjugate. Chromatography of these same samples on TLC results in a ninhydrin positive test for the 7,8-diol spot which indicates that the S metabolite is part of the 7,8-diol spot. The HPLC data indicate that the 7,8-diol TLC spot contain approximately 3-4% 7,8-diol. The rest of the percentage in the 7,8-diol spot is attributed to the S metabolite as derived from HPLC data. The S metabolite is still under investigation.

The low levels of quinones, in particular the 5,5-quinone, as measured by HPLC and TLC methods are indicative of little oxidation due to workup procedures. In addition to retention times corrected fluorescence spectra of concentrated extracts of 50 ml of blood were chromatographed on HPLC and the various metabolite fractions were collected and their fluorescence spectra were compared with known standards 9,10-, 7,8- and 4,5-diols and the 90 and 3-OH. The quinones and 7-OH were not identified by this method. The 6-OH and 4,5-epoxide are shown to indicate relative retention times of these metabolites.

TABLE 3.

BaP STANDARDS ON tlc

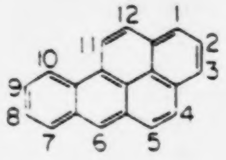
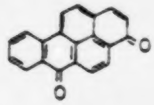
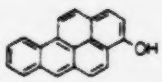
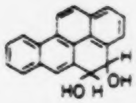
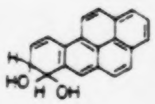
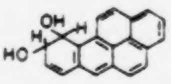
Compound		Rf Value
	B(a)P	0.88
	3,6-dione	0.78
	3-hydroxy	0.48
	4,5-dihydrodiol (P_2)	0.24
	7,8-dihydrodiol (Baseline)	0.03
	9,10-dihydrodiol (P_1)	0.16

FIGURE 9

HPLC of BaP Standard Control

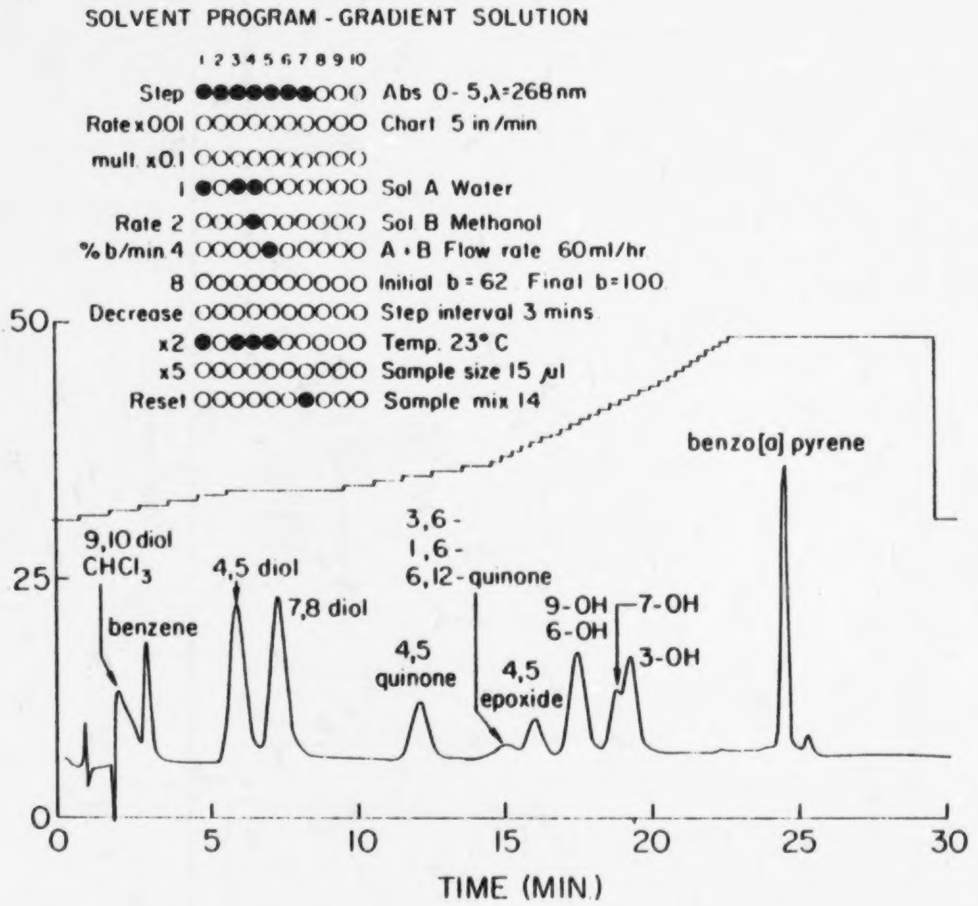
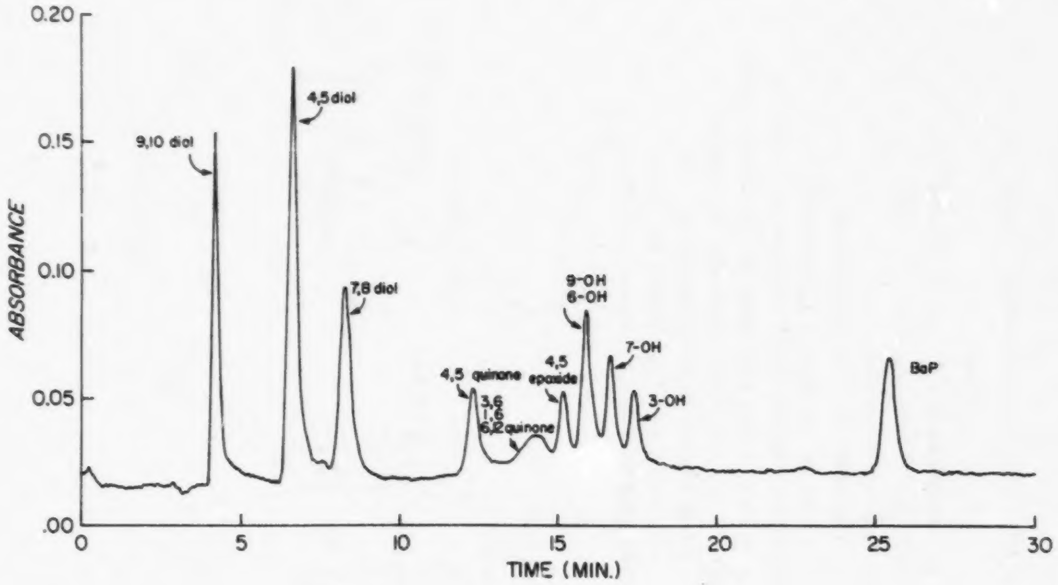


FIGURE 10

HPLC Chromatogram using HiBar II Reverse Column

Column - 10 u particle size, 25 cm x 4.6 mm



E. RESULTS

1. Effects of Enzyme Inducers

In Table 4 the various pretreatments used are listed. Table 5 shows the influence of enzyme inducers on the total metabolite appearance in the blood. IP pretreatment with Pheno. does not significantly influence the total rate of metabolism of BaP in the IPL. In fact, there appears to be a decrease in rate when compared to its control. BaP given IT or IP significantly induces BaP metabolism over control; BaP_{IP} is approximately 3 times the corn oil control while BaP_{IT} is approximately 5 times the non-pretreated control. Corn oil was found to increase the total rate of metabolism about twofold over the nonpretreated control while 3MC was found not to be significantly different from the corn oil control. It is perhaps interesting to note that the rate of BaP_{IT} plus the corn oil rate is approximately equivalent to BaP_{IP}.

a. Influence of BaP Pretreatment

BaP pretreatment IP or IT causes a large increase in the rate of metabolism of BaP (Table 6). This is due to the fact that BaP will induce the P_1450 (P448) enzyme system (64). The metabolic profile shows a marked increase in the 9,10-dihydrodiol for BaP_{IP} or IT pretreatment while there is a significant decrease in the monohydroxylated and diones for BaP_{IT} from the control and BaP_{IP} pretreatment. The nonextractables for the control and BaP_{IT} are comparable while the value for BaP_{IP} is considerably smaller.

These data suggest that even though BaP is given by two different routes of administration the rates of metabolism are significantly higher than the control in both cases and similar enzyme systems are induced. In one case the enzyme levels are increased in whole animal but especially in the liver 24 hrs. later, while in the second case the enzyme levels are increased specifically in the lung over a six week period. However, the metabolic patterns do show differences in that larger amounts of phenols and diones are formed, and therefore, less nonextractable materials for BaP_{IP} pretreatment. The nonextractables may indicate that there is less material available for binding, i.e. dihydrodiols or epoxides.

HPLC supports the TLC data (Tables 7,8, & 9) (25,55). Four BaP Control experiments in Table 7, show that the TLC is very comparable to the HPLC. In addition the 7,8-dihydrodiol and the S metabolite (which appears after BaP on HPLC) co-chromatograph on TLC and are comparable. The chemical characterization of the S metabolite is unknown at present; it is fluorescent and ninhydrin positive.

Data for BaP$_{IT}$ and BaP$_{IP}$ pretreatment experiments in Tables 8 and 9 show comparable results for HPLC and TLC. It should be noted, however, that these are 60 minute time points and not values from a slope. There are some discrepancies in the diones and phenol values but that is to be expected considering the differences in techniques.

TABLE 4.

PRETREATMENT REGIMEN OF ENZYME INDUCERS

Group	Dose (Amt/kg)	Time of Administration (hr) Pre-Sacrifice
Control	-	-
Pb_{IP}	50 mg	72,48 and 24
Corn Oil_{IP}	3 ml	24
$3 - MC_{IP}$	20 mg	24
$B(a)P_{IP}$	20 mg	24
$B(a)P_{IT}$	10 mg	once/wk x 5, 24

IT - Intratracheal

IP - Intraperitoneal

TABLE 5.

INFLUENCE OF ENZYME INDUCERS ON TOTAL METABOLITE APPEARANCE IN THE BLOOD

Pretreatment	N	Total Rate of Appearance (ng/hr/g lung ± S.E.)
Control_{IP}	9	256 ± 38
Pb_{IP}	4	162 ± 22
Corn Oil_{IP}	5	518 ± 97*
3 - MC_{IP}	4	836 ± 343
B(a)P_{IP}	5	1718 ± 287**
B(a)P_{IT}	5	1290 ± 114**

IT - Intratracheal

IP - Intraperitioneal

* P = 0.05

** P = 0.01 by Student-Newman-Keuls Test

TABLE 6.

INFLUENCE OF ENZYME INDUCERS ON THE METABOLISM OF BENZO(a)PYRENE ON THE IPL RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	Control	BaP_{IT}	BaP_{IP}
IPL	BaP	BaP	BaP
No. of Animals	9	5	5
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	256 ± 38	1290 ± 114[a]	1718 ± 287[a]
Metabolic pattern in blood (% ± S.E.)			
7,8-dihydrodiol	6.5 ± 0.9	5.4 ± 2.6	8.4 ± 0.7
9,10-dihydrodiol	14.5 ± 3.4	32.8 ± 8.6[b]	27.9 ± 4.5
4,5-dihydrodiol	3.4 ± 0.6	1.8 ± 1.1	3.8 ± 0.9
Monohydroxylated	9.7 ± 1.1	5.9 ± 2.3[b]	13.0 ± 3.5
Diones	10.7 ± 1.8	2.8 ± 2.1[b]	10.6 ± 1.8
Nonextractables	54.5 ± 5.4	51.8 ± 6.3	36.3 ± 4.7

a. P = 0.01

b. P = 0.05 by Student-Newman-Keuls Test

All three columns compared to each other. All metabolites separated by tlc.

TABLE 7.

COMPARISON HPLC AND tlc DATA

Pretreatment	-
IPL	BaP
No. of Animals	4

	HPLC	tlc
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	334±40	364±45
Metabolic pattern in blood (% ± S.E.)[a]		
9,10-dihydrodiol	20.1±6.4	18.3±7.5
4,5-dihydrodiol	2.8±0.4	2.2±1.0
7,8-dihydrodiol	2.7±0.6	10.3±2.7
4,5-quinone	1.4±0.6	3.6±0.8
1,6-6,12-3,6-quinone	2.3±0.6	
4,5 epoxide[b]	2.3±1.0	
9,6-OH	2.1±0.9	6.3±2.7
7-OH	2.6±0.3[c]	
3-OH		
S Metabolite	9.4±3.8	
Nonextractable	54.3±7.7	59.2±9.9

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[a] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[b] ^{14}C counts appear under peak.

[c] 7-OH and 3-OH collected together.

TABLE 8.

COMPARISION OF HPLC AND tlc DATA

Pretreatment	BaP_{IT}	
	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.)	1505 ± 157	1431 ± 153
Metabolite Pattern (% ± S.E.)[w]		
9,10-dihydrodiol	30.1 ± 5.1	37.7 ± 5.9
4,5-dihydrodiol	3.2 ± 0.2	1.4 ± 0.4
7,8-dihydrodiol	3.6 ± 0.3	7.6 ± 1.5
4,5-quinone	1.5 ± 0.1	3.3 ± 1.0
3,6-quinone	3.2 ± 0.9	
4,5-epoxide[y]	5.8 ± 0.8	
9 & 6-OH	2.5 ± 0.4	6.7 ± 1.3
3 & 7-OH	3.7 ± 0.2	
S metabolite	5.1 ± 0.8	
nonextractable	41.1 ± 4.4	43.3 ± 4.5

[w] Metabolite pattern values expressed as % of appearance of all metabolites in blood at 60 minutes ± standard error.

[y] ^{14}C counts appear under this peak.

n = 4

TABLE 9.

COMPARISON OF HPLC AND tlc DATA

Pretreatment	BaP$_{IP}$	
IPL	BaP	
No. of Animals	5	
	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.)	1624 ± 227	1741 ± 378
Metabolite Pattern (% ± S.E.)[w]		
9,10-dihydrodiol	30.4 ± 6.0	27.5 ± 4.2
4,5-dihydrodiol	3.8 ± 0.5	4.1 ± 0.7
7,8-dihydrodiol	3.1 ± 0.5	8.7 ± 1.2
4,5-quinone	1.4 ± 0.3	10.6 ± 1.9
3,6-,1,6-,6,12-quinone	2.8 ± 0.5	
4,5-epoxide[y]	2.0 ± 0.5	
9 & 6-OH	3.9 ± 1.5	13.0 ± 3.5
3 & 7-OH	4.2 ± 1.0	
nonextractable	37.0 ± 4.4	34.0 ± 4.1
S metabolite	11.7 ± 3.2	

[w] Metabolite pattern value expressed as % of appearance of all metabolites in blood at 60 minutes ± standard error.

[y] ^{14}C counts appear under this peak.

b. Influence of Various P_1450 Enzyme Inducers

BaP is a better enzyme inducer than 3MC, as indicated by the total rate of metabolism (Table 10). The metabolic pattern indicates that both 3MC and BaP have similar profiles which are different from the corn oil control. There appears to be more 9,10-dihydrodiol, diones, and phenols and less nonextractables than the control corn oil. HPLC data for corn oil (Table 11) at 60 minutes suggests that the 7,8-dihydrodiol may be mostly the unknown S metabolite (see methods).

These data suggest that most of metabolites formed by P_1450 enzyme inducers have similar metabolic pathways and that more polar material is excreted into the blood stream. The metabolic turnover rate is faster and, therefore, the increased amount of epoxides that are formed as intermediates rearrange or isomerize to phenols and oxidize to quinones or hydrate to the 9,10-dihydrodiols by epoxide hydrase action. This shunting to other pathways produces a smaller percentage of bound material (nonextractables) (64).

c. Influence of P_1450 Enzyme Inducer on BaP Metabolism

Phenobarbital does not induce the aryl hydrocarbon hydroxylase enzyme system (P_1450) as seen in Table 12 (64). Corn oil, however, is significantly different from the control which indicates that corn oil does induce the enzyme system. The metabolic profiles for corn oil and the control are similar, showing only slightly fewer phenols and quinones for corn oil. Phenobarbital, on the other hand, produces an increased quinone formation which is consistent with lack of P_1450 induction.

d. Distribution of BaP and Metabolites in Tissues at 180 Minutes in the IPL Following BaP Pretreatment

there is less unmetabolized BaP remaining in the IPL with either BaP_{IT} or BaP_{IP} pretreatment than their appropriate controls. This is consistent with an increase in enzyme activity in each case due to the BaP (Table 13 and 14).

There is a relative increase in the metabolic material and a decrease in BaP in the blood at 180 minutes after BaP_{IP} or BaP_{IT} pretreatment compared to their appropriate controls. This is reflected in a large increase in the 9,10-dihydrodiol and the nonextractables. Smaller increases are observed for the phenols and quinones after BaP_{IP} pretreatment.

Similar results are obtained for the distribution in the lung. There is a relative increase in the metabolite material and a decrease in BaP in the lung at 180 minutes after BaP pretreatment compared to controls. This is reflected in a large increase in the nonextractables after IT pretreatment and an increase in nonextractables and phenols after IP pretreatment. There is very little metabolite in the macrophage under either pretreatment. Also, there is a decrease in the relative amount of BaP in BaP pretreatments compared to controls. This is reflected in a slight increase in phenols, quinones, and nonextractables in both cases.

The relative amounts of metabolite in trachea bronchi decrease slightly after BaP_{IT} pretreatment and increase slightly after BaP_{IP} pretreatment as compared to their controls. There is a large decrease in the relative amounts of BaP given in the trachea bronchi. These data are reflected in an increase in nonextractables for IT pretreatment, and an increase in the dione fraction and decrease in nonextractables for IP pretreatment. Lastly, the increase in the relative amounts of metabolite in the washout are indicated by large increases in the nonextractable material.

Summary

With the addition of BaP_{IP} or BaP_{IT} as a pretreatment, there are large increases in rate of appearance of metabolites in the blood and large changes in the distribution of BaP and its metabolites in the blood and lung. With concomitant increases in total metabolite in blood and lung, there are corresponding decreases of BaP in these tissues. These major changes are consistent with increases in nonextractables in blood and lung and with increases in 9,10-dihydrodiol in the blood. Small increases and decreases of BaP and metabolite in the macrophage, washout, and trachea bronchi are also observed in relative changes in nonextractables and quinones or phenols. This suggests that as more intermediate epoxides are formed at a faster rate, they are converted into conjugates of BaP, or bound to macromolecules. Under both pretreatments, as the pathway becomes saturated, the epoxide hydrase converts the 9,10-epoxide to the 9,10-dihydrodiol and excretes it into the blood. Rearrangements and/or isomerization converts some intermediates to phenols and quinones after BaP_{IP} pretreatment. This last statement also suggests differences due to route of administration which in turn reflects differences in enzyme activities.

TABLE 10.

INFLUENCE OF ENZYME INDUCERS ON THE METABOLISM OF BENZO(a)PYRENE ON THE IPL RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	Corn Oil$_{IP}$	3-MC$_{IP}$	BaP$_{IP}$
IPL	BaP	BaP	BaP
No. of Animals	5	4	4
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.	518 ± 97	836 ± 343	1718 ± 287[a]
Metabolic pattern in blood (% ± S.E.)			
7,8-dihydrodiol	17.9 ± 4.9	5.4 ± 1.0[a]	8.4 ± 0.7[a]
9,10-dihydrodiol	15.4 ± 4.9	33.3 ± 2.0[b]	27.9 ± 4.5
4,5-dihydrodiol	1.2 ± 0.5	4.3 ± 0.8	3.8 ± 0.9
Monohydroxylated	2.7 ± 1.1	14.0 ± 2.4[a]	13.0 ± 3.5[b]
Diones	6.5 ± 2.9	11.7 ± 3.8	10.6 ± 1.8
Nonextractables	56.3 ± 7.8	32.6 ± 2.7	36.3 ± 4.7

a. P - 0.01

b. P = 0.05 by Student-Newman-Keuls Test

All three columns compared to each other. All metabolites separated by tlc.

TABLE 11.

COMPARISON OF HPLC AND tlc DATA

Pretreatment	Corn Oil$_{IP}$	
IPL	BaP	
No. of Animals	5	
	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.)	581 ± 63	647 ± 90
Metabolite Pattern (% ± S.E.)[w]		
9,10-dihydrodiol	16.5 ± 5.5	10.7 ± 2.5
4,5-dihydrodiol	2.8 ± 1.1	1.3 ± 0.5
7,8-dihydrodiol	2.1 ± 0.6	25.4 ± 9.7
4,5-quinone	1.8 ± 0.4	8.9 ± 4.0
3,6-,1,6-,6,12-quinone	5.9 ± 2.0	
4,5-epoxide[y]	1.9 ± 0.5	
9 & 6-OH	2.8 ± 0.8	2.7 ± 1.0
3 & 7-OH	2.4 ± 0.6	
nonextractable	52.3 ± 9.4	51.0 ± 11.7
S metabolite	10.9 ± 2.0	

[w] Metabolite pattern value expressed as % of appearance of all metabolites in blood at 60 minutes ± standard error.

[y] ^{14}C counts appear under this peak.

TABLE 12.

INFLUENCE OF ENZYME INDUCERS ON THE METABOLISM OF BENZO(a)PYRENE ON THE IPL RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	Control	Pheno$_{IP}$	Corn Oil$_{IP}$
IPL	BaP	BaP	BaP
No. of Animals	9	4	5
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	256 ± 38	162 ± 22	518 ± 97[b]
Metabolic pattern in blood (% ± S.E.)			
7,8-dihydrodiol	6.5 ± 0.9	6.9 ± 2.0	17.9 ± 4.9[a]
9,10-dihydrodiol	14.5 ± 3.4	4.8 ± 1.0[c]	15.4 ± 4.9
4,5-dihydrodiol	3.4 ± 0.6	3.1 ± 1.0	1.2 ± 0.5[b]
Monohydroxylated	9.7 ± 1.1	6.3 ± 1.1[c]	2.7 ± 1.1[a]
Diones	10.7 ± 1.8	21.9 ± 4.4[b]	6.5 ± 2.9
Nonextractables	54.5 ± 5.4	59.1 ± 6.5	56.3 ± 7.8

a. P = 0.01

b. P = 0.05

c. P = 0.10 (by Student-Newman-Keuls Test)

All three columns compared to each other. All metabolites separated by tlc.

TABLE 13.

ENZYME INDUCERS

% OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE
AT 180 MINUTES ± S.E.

Pretreatment	None	BaP_{IT}	Corn Oil	BaP_{IP}
IPL	BaP	BaP	BaP	BaP
No. of Animals	3	3	2	2
% of Unmetabolized BaP	65.3 ± 4.3	21.9 ± 3.1	60.9 ± 3.4	23.5 ± 2.5
% of Total Compound as Metabolite in Tissue				
Blood	15.5 ± 5.2	41.5 ± 7.5	14.5 ± 2.5	39.7 ± 17.9
TB	3.7 ± 1.2	1.3 ± 0.5	3.4 ± 0.1	5.9 ± 4.6
MAC	0.3 ± 0.1	0.8 ± 0.2	0.9 ± 0.3	0.6 ± 0.5
WO	3.3 ± 2.3	6.3 ± 0.5	2.6 ± 0.4	8.9 ± 7.2
Lung	12.0 ± 1.9	28.0 ± 1.4	17.6 ± 0.8	21.4 ± 8.0
% of Total Compound as BaP in Tissue				
Blood	12.7 ± 1.4	1.9 ± 0.2	15.8 ± 5.6	1.5 ± 1.0
TB	10.4 ± 2.7	1.5 ± 0.5	5.4 ± 1.8	6.2 ± 3.0
MAC	8.7 ± 7.4	4.7 ± 0.6	3.8 ± 0.4	1.0 ± 0.7
WO	2.5 ± 1.1	2.4 ± 0.6	5.2 ± 3.9	2.1 ± 0.4
Lung	30.9 ± 3.6	11.5 ± 3.0	30.6 ± 4.7	12.6 ± 0.7

TABLE 14.

% DISTRIBUTION PATTERN OF BaP + METABOLITES IN EACH TISSUE

LUNG^x

Pretreatment	Control	BaP_{IT}	Corn Oil	BaP_{IP}
7,8-dihydrodiol	1.2±0.1	5.4±0.7	3.5±0.1	4.5±1.3
9,10-dihydrodiol	2.9±1.5	2.7±0.6	1.0±0.3	5.4±2.7
4,5-dihydrodiol	0.9±0.4	0.9±0.3	0.3±0.0	0.6±0.0
monohydroxylated	10.1±0.6	3.5±1.4	2.2±1.5	11.9±6.1
diones	1.1±0.3	3.7±0.7	1.2±0.2	4.9±1.4
nonextractable	5.3±0.9	43.9±4.6	13.2±1.9	26.7±0.5
BaP	78.5±2.7	39.8±7.4	78.5±3.4	45.9±10.9

^{x}Based on % total activity at 180 minutes in each tissue ± S.E.

Control, BaP_{IT}, Corn Oil and BaP_{IP} - 3,3,2,2 animals respectively.

Control and BaP_{IT} comparable.

Corn Oil and BaP_{IP} comparable.

(continued)

TABLE 14.

(continued)

180 BLOOD[x]

Pretreatment	Control	BaP_{IT}	Corn Oil	BaP_{IP}
7,8-dihydrodiol	2.8±0.5	6.4±1.4	2.3±0.1	6.5±0.6
9,10-dihydrodiol	6.5±3.8	17.4±2.5	2.3±1.1	14.8±5.9
4,5-dihydrodiol	1.2±0.7	1.9±1.2	0.8±0.5	1.0±0.3
monohydroxylated	2.5±0.9	3.3±0.6	2.8±0.7	9.1±5.4
diones	1.3±0.1	1.9±0.4	1.1±0.3	8.9±3.4
nonextractable	18.6±2.5	61.5±2.5	21.9±4.2	55.4±16.5
BaP	67.0±6.5	7.6±1.4	68.9±4.1	4.2±1.4

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Control, BaP_{IT}, Corn Oil and BaP_{IP} - 3,3,2,2 animals respectively.

Control and BaP_{IT} comparable.

Corn Oil and BaP_{IP} comparable.

(continued)

TABLE 14.

(continued)

MACROPHAGE[x]

Pretreatment	Control	BaP_{IT}	Corn Oil	BaP_{IP}
7,8-dihydrodiol	1.4±0.4	1.4±0.3	2.6±0.9	2.6±0.6
9,10-dihydrodiol	0.9±0.2	0.9±0.2	4.2±3.0	2.3±0.6
4,5-dihydrodiol	0.2±0.2	0.1±0.0	0.0±0.0	0.7±0.1
monohydroxylated	0.9±0.3	1.3±0.6	1.0±0.3	5.6±2.9
diones	0.5±0.2	2.4±1.0	0.4±0.1	8.0±7.6
nonextractable	1.9±1.0	3.5±0.9	2.2±0.7	8.4±4.5
BaP	9.42±2.2	90.3±2.5	89.6±4.1	72.5±5.9

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Control, BaP_{IT}, Corn Oil and BaP_{IP} - 3,3,2,2 animals respectively.

Control and BaP_{IT} comparable.

Corn Oil and BaP_{IP} comparable.

(continued)

TABLE 14.

(continued)

WASHOUT[x]

Pretreatment	Control	BaP_{IT}	Corn Oil	BaP_{IP}
7,8-dyhydrodiol	5.0±1.8	5.5±1.8	4.5±1.5	3.5±0.8
9,10-dihydrodiol	10.8±8.3	7.1±1.2	2.5±1.1	7.3±4.8
4,5-dihydrodiol	0.8±0.6	0.6±0.4	0.4±0.1	0.7±0.1
monohydroxylated	2.7±2.0	3.8±1.1	1.6±1.1	7.4±1.4
diones	2.1±1.3	3.8±1.3	0.7±0.1	2.1±0.0
nonextractable	17.5±6.6	39.6±4.6	18.9±16.3	39.8±19.7
BaP	61.0±7.7	39.6±2.2	71.5±15.5	39.2±26.6

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Control, BaP_{IT}, Corn Oil and BaP_{IP} - 3,3,2,2 animals respectively.

Control and BaP_{IT} comparable.

Corn Oil and BaP_{IP} comparable.

(continued)

TABLE 14.

(continued)

TRACHEA BRONCHI[x]

Pretreatment	Control	BaP_{It}	Corn Oil	BaP_{IP}
7,8-dyhydrodiol	0.8±0.2	4.4±0.7	1.5±0.9	2.3±0.3
9,10-dihydrodiol	2.2±1.2	3.4±0.8	0.7±0.7	3.1±0.8
4,5-dihydrodiol	0.9±0.5	0.5±0.2	0.5±0.2	0.7±0.6
monohydroxylated	3.4±1.8	1.4±0.7	1.1±0.0	4.0±2.2
diones	2.1±1.4	3.2±2.6	0.7±0.7	11.9±6.2
nonextractable	12.5±4.1	24.8±11.5	17.4±10.7	62.4±2.6
BaP	79.4±7.4	62.2±10.1	78.1±8.2	65.5±11.4

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Control, BaP_{IT}, Corn Oil and BaP_{IP} - 3,3,2,2 animals respectively.

Control and BaP_{IT} comparable.

Corn Oil and BaP_{IP} comparable.

2. Effects of Particulate

a. Influence of Particulate Administered to IPL on BaP Metabolism

1) Rate of Metabolism

The crude air particulate (or ferric oxide) acts to inhibit the rate of metabolism (Table 15) of BaP slightly. This can be due to either a biochemical effect or a physical effect, i.e. particulate engulfed more readily by macrophages, and therefore, rendering the BaP less biologically available for metabolism over the time of study. However, there is not a significant differency between CAP (or ferric oxide) and BaP and BaP alone on the rate of metabolism of BaP on the IPL. The metabolic pattern shows a marked increase in 7,8- and 9,10-diols and a decrease in the nonextractable or polar and monohydroxy and quinone metabolites. This indicates an inhibition of the particulate on the multiple enzymes (17,28,37,48,71) which is consistent with diol epoxide formation and a decrease in hydroxylation and/or isomerization and polar (nonextractable) conjugation pathways. The mechanism for the decrease in the nonextractable or polar metabolite is not understood at present.

The HPLC data generally supports the TLC data (Table 16). There are a few differences that may be due to 1) the techniques involved and 2) the values at 60 minutes on HPLC versus the values obtained from the slope of the curve for TLC (25,55).

2) Distribution of BaP and Its Metabolites in Tissue at 180 Minutes

There is a relative decrease of BaP in the blood at 180 minutes compared to control (Table 17 & 18), while the lung and trachea bronchi show a relative increase in metabolite and a decrease in BaP. These results are consistent with an increase in the nonextractables. The macrophage and washout both show increases in the relative amounts of the BaP metabolite content. The only changes that are observed in their metabolite profiles are the 7,8 and 9,10-diols and the nonextractables for the washout fraction.

Summary

The particulate inhibits the rate of appearance of metabolites of BaP observed in the blood. This can be explained by the relative increase of BaP and metabolites in the macrophage and washout with corresponding decrease of BaP in the lung and trachea bronchi. The BaP adsorbed particulate can be engulfed more readily by macrophages and, therefore, BaP is less biologically available for metabolism or is leached more slowly from the lung. However, at the end of 180 minutes the relative amount of BaP metabolized by CAP plus BaP is greater than the control. This is consistent with an increase of metabolite in lung and trachea bronchi, as well as washout and macrophage. This then might suggest that the CAP, which contains a variety of PAHs and metals, might induce the enzyme system slightly after a period of time for equilibration or that the macrophage metabolize BaP but dealy the excretion of these metabolites into the blood.

TABLE 15.

INFLUENCE OF PARTICULATES ADMINISTERED TO IPL OR BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	-	-
IPL	BaP	BaP + CAP[a]
No. of Animals	9	5
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	256 ± 38	156 ± 42
Metabolic pattern in blood (% ± S.E.)[b]		
7,8-dihydrodiol	6.6 ± 0.9	19.1 ± 4.4[c]
9,10-dihydrodiol	15.4 ± 4.0	28.3 ± 7.9
4,5-dihydrodiol	3.3 ± 0.6	3.0 ± 1.3
Monohydroxylated	9.7 ± 1.1	5.1 ± 1.4[e]
Diones	10.6 ± 1.8	5.2 ± 2.6
Nonextractable	54.4 ± 5.4	39.3 ± 13.8

All metabolites separated by tlc.

[a] 1 mg/kg

[b] Metabolite pattern expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[c] $P = 0.01$

[d] $P = 0.05$

[e] $P = 0.1$ (Student-Newman-Keuls Test)

TABLE 16.

COMPARISON OF HPLC AND tlc DATA

Pretreatment	None	
IPL	BaP + CAP	
No. of Animals	5	
	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.)	208 ± 18	203 ± 34
Metabolite Pattern (% ± S.E.)[w]		
9,10-dihydrodiol	14.9 ± 6.8	25.0 ± 7.5
4,5-dihydrodiol	2.3 ± 0.9	1.8 ± 1.6
7,8-dihydrodiol	2.8 ± 0.6	11.2 ± 2.8
4,5-quinone	3.0 ± 0.7	
3,6-,1,6-,6,12-quinone	3.4 ± 1.0	2.8 ± 1.1
4,5-epoxide[y]	1.4 ± 0.5	
9 & 6-OH	1.9 ± 0.7	4.2 ± 1.4
3 & 7-OH	2.8 ± 0.9	
nonextractable	50.6 ± 8.2	54.9 ± 11.7
S metabolite	16.8 ± 3.0	

[w] Metabolite pattern value expressed as % of appearance of all metabolites in blood at 60 minutes ± standard error.

[y] ^{14}C counts appear under this peak.

TABLE 17.

INFLUENCE OF PARTICULATE ON THE IPL
% OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE AT 180 MINUTES ± S.E.

Pretreatment	-	-
IPL	BaP	BaP + CAP
No. of Animals	3	2
% Unmetabolized BaP	65.3 ± 4.3	40.0 ± 26.3
% of Total Compound as Metabolite in Tissue		
Blood	15.5 ± 5.3	16.6 ± 6.7
TB	3.7 ± 1.2	6.2 ± 2.8
MAC	0.3 ± 0.1	1.9 ± 1.3
WO	3.3 ± 2.3	13.2 ± 3.3
Lung	12.0 ± 1.9	22.1 ± 9.1
% of Total Compound as BaP in Tissue		
Blood	12.7 ± 1.4	1.0 ± 0.8
TB	10.4 ± 2.7	2.3 ± 1.0
MAC	8.7 ± 7.4	17.4 ± 12.2
WO	7.5 ± 1.1	10.9 ± 9.2
Lung	30.9 ± 3.6	8.5 ± 2.5

TABLE 18.

% DISTRIBUTION PATTERN OF BaP + METABOLITES IN EACH TISSUE

	LUNG[x]		180 BLOOD[x]	
Pretreatment	None	None	None	None
IPL	BaP	BaP+CAP	BaP	BaP+CAP
7,8-dihydrodiol	1.2±0.1	6.1±0.5	2.8±0.5	8.0±5.8
9,10-dihydrodiol	2.9±1.5	3.2±0.2	6.5±3.8	5.6±3.4
4,5-dihydrodiol	0.9±0.4	1.3±0.2	1.2±0.7	0.9±0.6
monohydroxylated	10.1±0.6	3.4±0.7	2.5±0.9	2.2±1.8
diones	1.1±0.3	1.3±1.0	1.3±0.1	1.5±1.3
nonextractable	5.3±0.9	24.7±2.1	18.6±2.5	[x]59.7±33.7
BaP	78.5±2.7	60.0±3.2	67.0±6.5	[x]22.1±20.8

[x] Based on % of total activity at 180 minutes in each tissue ± S.E. Number of animals 3 & 2 respectively.

(continued)

TABLE 18.

(continued)

	MACROPHAGE[x]		WASHOUT[x]	
Pretreatment	None	None	None	None
IPL	BaP	BaP+CAP	BaP	BaP+CAP
7,8-dihydrodiol	1.4±0.4	1.5±0.5	5.0±1.8	[x]28.4±19.6
9,10-dihydrodiol	0.9±0.2	0.3±0.2	10.8±8.3	5.3±0.5
4,5-dihydrodiol	0.2±0.2	0.1±0.0	0.8±0.6	0.9±0.3
monohydroxylated	0.9±0.3	0.2±0.1	2.7±2.0	3.2±2.1
diones	0.5±0.2	0.2±0.1	2.1±1.3	2.4±0.6
nonextractable	1.9±1.0	1.4±0.3	17.5±6.6	0.00
BaP	94.2±2.2	96.3±1.3	61.0±7.7	[x]59.9±22.1

[x] Based on % of total activity at 180 minutes in each tissue ± S.E. Number of animals 3 & 2 respectively.

(continued)

TABLE 18.

(continued)

	TRACHEA BRONCHI[x]	
Pretreatment	None	None
IPL	BaP	BaP+CAP
7,8-dihydrodiol	0.8±0.2	3.5±1.2
9,10-dihydrodiol	2.2±1.2	2.6±1.7
4.5-dihydrodiol	0.9±0.5	0.4±0.3
monohydroxylated	3.4±1.8	0.3±0.3
diones	2.1±1.4	3.5±1.5
nonextractable	12.5±4.1	[x]26.7±16.1
BaP	79.4±7.4	63.1±21.0

[x] Based on % of total activity at 180 minutes in each tissue ± S.E. Number of animals 3 & 2 respectively.

b. Influence of BaP Pretreatment and Particulate Administered on IPL on BaP Metabolism

1) Rate of Metabolism

BaP pretreatment increases significantly the rate of metabolism as compared to the corn oil control. BaP pretreatment and CAP or ferric oxide in perfusion, however, decrease the rate of metabolism significantly as compared to BaP_{IP} control (Table 19) and is comparable to the corn oil control. The metabolic effect of the BAP may be due to an increase in the rate of action of the macrophage or the slow physical release of BaP. The rate of metabolism due to the particulate is not influenced by pretreatment with enzyme inducers. The pattern due to the particulate indicates a trend toward glutathione transferase and/or epoxide hydrase and away from isomerization and/or polyhydroxylation. This is reflected in an increase in the polar or nonextractable and 7,8- and 9,10-diol metabolites versus diones and monohydroxylated metabolites. Secondly, the particulate increases the nonextractable or polar material versus the diols, i.e. possible BaP conjugation versus epoxide hydrase and hydration. Lastly, the metabolite profile for BaP_{IP} followed by BaP plus CAP on the IPL is very similar to the corn oil pretreatment alone, which further indicates the effect of CAP on this type of pretreatment. As indicated, HPLC data supports tlc data (Table 20) rather well. The 7,8-dihydrodiol and the S metabolite on HPLC chromatograph together on tlc (25,55).

2) Distribution of BaP and Its Metabolites in Tissue at 180 Minutes

There is a large increase in the relative amount of metabolites and a corresponding relative decrease of BaP in both the blood and lung for the BaP_{IP} pretreatment compared to its control (Fig. 21 & 22). This also is consistent with a decrease in the relative amount of total BaP left in perfusion at 180 minutes. The results for the BaP_{IP} pretreatment followed by BaP plus CAP experiments are similar to BaP_{IP} pretreatment experiments. The results for the blood are reflected in an increase in the excretion of the nonextractables, as well as the 9,10-dihydrodiol and phenols. There are some differences, however, between the two experimental groups in the lung; there is a larger amount of phenol and nonextractable in BaP pretreatment experiments than in the CAP experimental group. This can be attributed to the fact that the BaP may be more readily available for metabolism than in the case involving CAP plus BaP.

This last fact is consistent with the relative increase in quinones, phenols and nonextractables and the decrease in BaP in the macrophage for BaP_{IP} pretreatment. These results are not seen when CAP is introduced in the system. CAP would appear to enhance the action of the macrophage.

There appears to be minor changes in the relative amounts of BaP and metabolite in washout and trachea bronchi for both experimental groups. In both cases the relative amounts of nonextractables are increased in the washout with a larger increase observed when CAP is introduced. In the trachea bronchi there is a decrease in the nonextractables and a relative increase in phenols for BaP_{IP} pretreatment compared to its control. When CAP is introduced a higher percentage of BaP is found which is consistent with the fact that BaP is not available for metabolism or that clearance of BaP is slowed by CAP.

TABLE 19.

INFLUENCE OF BaP PRETREATMENT AND PARTICULATE ADMINISTERED ON IPL ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment IPL No. of Animals	Corn Oil$_{IP}$[a] BaP 5	BaP$_{IP}$[b] BaP 5	BaP$_{IP}$[b] BaP + CAP[c] 5
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	466 ± 94[e]	1718 ± 287	414 ± 95[e]
Metabolic pattern in blood (% ± S.E.)[d]			
7,8-dihydrodiol	17.9 ± 0.9[f]	8.4 ± 0.7	11.8 ± 0.6[a]
9,10-dihydrodiol	15.3 ± 4.9	27.9 ± 4.5	18.6 ± 2.7
4,5-dihydrodiol	1.2 ± 0.5[g]	3.8 ± 0.9	2.6 ± 1.6
Monohydroxylated	2.7 ± 1.1[g]	13.0 ± 3.5	3.5 ± 1.0[g]
Diones	6.5 ± 2.9	10.6 ± 1.8	2.3 ± 0.6[e]
Nonextractable	56.3 ± 7.8	36.3 ± 4.7	61.1 ± 3.6[g]

Columns 1 & 3 compared with column 2. All metabolites separated by tlc.

[a] 3 ml/kg.

[b] 20 mg/kg (24 hrs. before sacrifice).

[c] 1 mg/kg.

[d] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[e] P = 0.01

[f] P = 0.1

[g] P = 0.05 (Student-Newman-Keuls Test)

TABLE 20.

COMPARISON OF HPLC AND tlc DATA

Pretreatment	$\mathrm{BaP}_{\mathrm{IP}}$
IPL	BaP + CAP
No. of Animals	4

	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.)	424 ± 88	458 ± 99
Metabolite Pattern (% ± S.E.)[w]		
9,10-dihydrodiol	16.6 ± 3.2	20.1 ± 2.5
4,5-dihydrodiol	3.1 ± 0.4	2.9 ± 1.7
7,8-dihydrodiol	2.2 ± 0.5	13.5 ± 2.9
4,5-quinone	2.3 ± 0.5	
3,6-,1,6-,6,12-quinone	2.0 ± 0.6	3.4 ± 1.7
4,5-epoxide[y]	0.8 ± 0.3	
9 & 6-OH	1.1 ± 0.5	3.4 ± 1.1
3 & 7-OH	2.2 ± 0.5	
nonextractable	60.6 ± 4.5	56.5 ± 5.1
S metabolite	9.0 ± 1.4	

[w] Metabolite pattern value expressed as % of appearance of all metabolites in blood at 60 minutes - standard error.

[y] ^{14}C counts appear under this peak.

TABLE 21.

INFLUENCE OF BaP PRETREATMENT
% OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE AT 180 MINUTES ± S.E.

Pretreatment IPL No. of Animals	Corn Oil BaP 2	BaP_{IP} BaP 2	BaP_{IP} BaP + CAP 2
% Unmetabolized BaP	60.9 ± 3.4	23.5 ± 2.5	25.2 ± 5.9
% of Total Compound as BaP in Tissue			
Blood	14.5 ± 2.5	39.7 ± 17.9	41.2 ± 14.7
TB	3.4 ± 0.1	5.9 ± 4.6	3.8 ± 1.4
MAC	0.9 ± 0.3	0.6 ± 0.5	0.7 ± 0.1
WO	2.6 ± 0.4	8.9 ± 7.2	4.4 ± 1.9
Lung	17.6 ± 0.8	21.4 ± 8.0	24.5 ± 14.2
% of Total Compound as BaP in Tissue			
Blood	15.8 ± 5.6	1.5 ± 1.0	1.4 ± 0.7
TB	5.4 ± 1.8	6.3 ± 3.0	5.3 ± 1.5
MAC	3.8 ± 0.4	1.0 ± 0.7	3.4 ± 0.5
WO	5.2 ± 3.9	2.1 ± 0.4	0.2 ± 0.0
Lung	30.6 ± 4.7	12.6 ± 0.7	14.8 ± 6.8

Column 1 compared to Column 2.

Column 3 compared to Column 2.

TABLE 22.

% DISTRIBUTION PATTERN OF BaP + METABOLITES IN EACH TISSUE

LUNG[x]

Pretreatment	Corn Oil$_{IP}$	BaP$_{IP}$	BaP$_{IP}$
IPL	BaP	BaP	BaP+CAP
7,8-dihydrodiol	3.5±0.1	4.5±1.3	2.6±0.0
9,10-dihydrodiol	1.0±0.3	5.4±2.7	4.5±3.8
4,5-dihydrodiol	0.3±0.0	0.6±0.0	0.7±0.7
monohydroxylated	2.2±1.5	11.9±6.1	0.9±0.6
diones	1.2±0.2	4.9±1.4	0.2±0.2
nonextractable	13.2±1.9	26.7±0.5	18.9±0.4
BaP	78.5±3.4	45.9±10.9	72.1±3.1

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Two (2) animals per experiment respectively.

Corn Oil$_{IP}$ compared to BaP$_{IP}$.

BaP$_{IP}$ & BaP + CAP on IPL compared to BaP$_{IP}$.

(continued)

TABLE 22.

(continued)

180 BLOOD[x]

Pretreatment	Corn Oil$_{IP}$	BaP$_{IP}$	BaP$_{IP}$
IPL	BaP	BaP	BaP+CAP
7,8-dihydrodiol	2.3±0.1	6.5±0.6	7.8±2.0
9,10-dihydrodiol	2.3±1.1	14.8±5.9	11.7±2.7
4,5-dihydrodiol	0.8±0.5	1.0±0.3	0±00
monohydroxylated	2.8±0.7	9.1±5.4	11.6±1.1
diones	1.1±0.3	8.9±3.4	1.2±0.2
nonextractable	21.9±4.2	55.4±16.5	56.2±1.6
BaP	68.9±4.1	4.2±1.4	11.5±1.7

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Two (2) animals per experiment respectively.

Corn Oil$_{IP}$ compared to BaP$_{IP}$.

BaP$_{IP}$ & BaP + CAP on IPL compared to BaP$_{IP}$.

(continued)

TABLE 22.

(continued)

MACROPHAGE[x]

Pretreatment	Corn Oil$_{IP}$	BaP$_{IP}$	BaP$_{IP}$
IPL	BaP	BaP	BaP+CAP
7,8-dihydrodiol	2.6±0.9	2.6±0.6	1.5±0.4
9,10-dihydrodiol	4.2±3.0	2.3±0.6	1.3±1.0
4,5-dihydrodiol	0.0±0.0	0.7±0.1	0.1±0.1
monohydroxylated	1.0±0.3	5.6±2.9	0.1±0.0
diones	0.4±0.1	8.0±7.6	0.3±0.2
nonextractable	2.2±0.7	8.4±4.5	2.3±0.7
BaP	89.6±4.1	72.5±5.9	94.4±1.9

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Two (2) animals per experiment respectively.

Corn Oil$_{IP}$ compared to BaP$_{IP}$.

BaP$_{IP}$ & BaP + CAP on IPL compared to BaP$_{IP}$.

(continued)

TABLE 22.

(continued)

WASHOUT[x]

Pretreatment	Corn Oil$_{IP}$	BaP$_{IP}$	BaP$_{IP}$
IPL	BaP	BaP	BaP+CAP
7,8-dihydrodiol	4.5±1.5	3.5±0.8	4.2±1.9
9,10-dihydrodiol	2.5±1.1	7.3±4.8	3.8±2.3
4,5-dihydrodiol	0.4±0.1	0.7±0.1	0.4±0.1
monohydroxlated	1.6±1.1	7.4±1.4	0.4±0.0
diones	0.7±0.1	2.1±0.0	1.5±0.8
nonextractable	18.9±16.3	39.8±19.7	68.8±7.0
BaP	71.5±15.5	39.2±26.6	20.8±6.8

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Two (2) animals per experiment respectively.

Corn Oil$_{IP}$ compared to BaP$_{IP}$.

BaP$_{IP}$ & BaP + CAP on IPL compared to BaP$_{IP}$.

(continued)

TABLE 22.

(continued)

TRACHEA BRONCHI[x]

Pretreatment	Corn Oil$_{IP}$	BaP$_{IP}$	BaP$_{IP}$
IPL	BaP	BaP	BaP+CAP
7,8-dihydrodiol	1.5±0.9	2.3±0.3	1.9±1.1
9,10-dihydrodiol	0.7±0.7	3.1±0.8	1.3±0.9
4,5-dihydrodiol	0.5±0.2	0.7±0.6	0.6±0.3
monohydroxylated	1.1±0.0	4.0±2.2	0.5±0.3
diones	0.7±0.7	11.9±6.2	0.8±0.7
nonextractable	17.4±10.7	12.4±2.6	9.7±2.0
BaP	78.1±8.2	65.5±11.4	85.1±1.4

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Two (2) animals per experiment respectively.

Corn Oil$_{IP}$ compared to BaP$_{IP}$.

BaP$_{IP}$ & BaP + CAP on IPL compared to BaP$_{IP}$.

Summary

When BaP is given IP to the whole animal, the rate of appearance of metabolites is increased significantly compared to its control. However, when CAP is introduced with BaP on the perfusion the rate of appearance of metabolites is decreased. The rate of appearance and the metabolite profile are similar to the corn oil pretreatment, which indicates that CAP negates the effect of pretreatment. However, as the perfusion proceeds through to 180 minutes, more BaP is metabolized, i.e. more BaP is available by leaching from CAP. In fact, the profiles are similar for the two experimental groups which suggests that after equilibration for a period of time, more of the BaP adsorbed on CAP is available for metabolism. This is reflected in larger amounts of nonextractables and 9,10-diol excreted into the blood with corresponding decreases of BaP. However, there are still some minor differences at 180 minutes in that slightly more BaP and less metabolites are found in the macrophage, lung, and trachea bronchi after introduction of CAP. With additional time, more BaP may become available for metabolism, and therefore, these differences could disappear. Lastly, it should be mentioned that the CAP inhibits induced enzymes but not the basal enzymes.

c. Influence of Particulate Pretreatment on BaP Metabolism

1) Rate of Metabolism

Pretreatment with particulate causes a significant increase in the rate of metabolism (Table 23) versus its control. This suggests that the particulate may influence enzyme activity. This, of course, is especially reflected in CAP pretreatment due to the presence of other PAHs in the mixture. The metabolite profile shows a decrease in the nonextractable or polar material and monohydroxy compounds and an increase in the 7,8- and 9,10-diol formation. This reflects an increase in epoxide hydrase activity and a decrease in polar (nonextractable) conjugation.

When BaP adsorbed on CAP is added as pretreatment, the CAP acts to greatly inhibit the enzyme inducing ability of the BaP (results, Section 1a). The rate of appearance of metabolites is increased, however, for this set of experiments compared to its control which indicates a combined effect of BaP and CAP. The metabolite profile shows a decrease in the 7,8- and 9,10-diol formation and a small increase in the nonextractable material. This reflects a slight decrease in epoxide hydrase activity and an increase in the nonextractable conjugation.

The HPLC supports the tlc data (Table 24 & 25) for CAP alone and BaP adsorbed on CAP as pretreatments (25,55).

2) Distribution of BaP and Its Metabolites in Tissues at 180 Minutes

CAP given as a pretreatment (based on one experiment at 180 minutes) to the whole animal increases the amount of metabolite found in tissues at 180 minutes (Table 26 & 27). There is a corresponding relative increase of metabolite found in the blood and lung and a relative decrease in BaP. This is reflected in an increase in the 9,10- and 7,8-diols and a decrease in the BaP content in both tissues. The nonextractables increase in the lung and decrease in the blood compared to the control. There are some small relative increases of metabolite in the trachea bronchi and macrophage and corresponding decreases of BaP in these tissues while there are slight relative decreases in metabolite and BaP in washout compared to the control. These data are consistent with significant increases of 7,8- and 9,10-diols and quinones in macrophage and washout, increases of the diones in the trachea bronchi, and a significant increase of the nonextractables in the washout. These data suggest that CAP given as a pretreatment acts as an enzyme inducer; an increase in the rate of metabolism of BaP is reflected in an increase of diol formation in the tissues of the lung and smaller increases in the nonextractables, diones and phenols.

When BaP adsorbed on CAP is added to the whole animal, the relative amount of metabolism does not change compared to CAP alone at 180 minutes. There is an increase in the relative amount of metabolite in the blood and a decrease in the lung while the BaP content does not change significantly. There is a corresponding decrease in the 9,10-diol and a large increase in the nonextractables. A significant relative increase of metabolite in the washout and relative decreases of metabolite in trachea bronchi and macrophage are observed for BaP adsorbed on CAP compared to CAP alone. This is consistent with decreases in the amounts of the 7,8-and 9,10-diols in macrophages and diones in the trachea bronchi with corresponding increases of BaP in both tissues. Amounts of phenols increased in the trachea bronchi. No such metabolite profile changes are present in the washout data. These data suggest that BaP adsorbed on CAP does not change significantly the relative amounts of metabolite and BaP in tissues at 180 minutes. However, the metabolite distribution has changed in that there is a decrease in the diol formation and an increase of nonextractables which suggests that BaP and CAP together act differently than when administered separately as a pretreatment.

Summary

CAP acts as an enzyme inducer to increase the rate of metabolism of BaP. BaP adsorbed on CAP also acts like an enzyme inducer by increasing the rate of metabolism, but the rate of metabolism is not the sum of the two individual rates. This indicates that the BaP is not leached readily from the CAP, i.e. BaP is not available for enzyme induction. Overall CAP or BaP (results, Section 1d) by itself increase the diol formation in the tissues with smaller increases or decreases in nonextractables. Together BaP and CAP decrease the diol formation and increase the nonextractables in the tissues.

TABLE 23.

INFLUENCE OF PARTICULATE PRETREATMENT ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	-	$\text{CAP}_{\text{IT}}{}^{a}$	$(\text{BaP + CAP})_{\text{IT}}{}^{a}$
IPL	BaP	BaP	BaP
No. of Animals	9	5	5
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	256 ± 37[c]	830 ± 100	1093 ± 153
Metabolite pattern in blood (% ± S.E.)[b]			
7,8-dihydrodiol	6.6 ± 0.9[d]	18.2 ± 5.6	11.2 ± 3.4
9,10-dihydrodiol	15.4 ± 4.0[d]	32.6 ± 4.3	25.9 ± 2.4
4,5-dihydrodiol	3.3 ± 0.6[d]	0.9 ± 0.5	4.9 ± 2.5
Monohydroxylated	9.7 ± 1.1[c]	3.4 ± 0.6	5.7 ± 0.9[e]
Diones	10.6 ± 1.8[e]	5.5 ± 1.8	7.0 ± 1.2
Nonextractable	54.4 - 5.4	39.4 - 8.0	45.2 - 4.3

Columns 1 & 3 compared to Column 2. All metabolites separated by tlc.

[a] 10 mg/kg, once a week x 5, BaP and/or CAP.

[b] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[c] $P = 0.01$

[d] $P = 0.05$

[e] $P = 0.10$ (Student-Newman-Keuls Test)

TABLE 24.

COMPARISON OF HPLC AND tlc DATA

Pretreatment IPL No. of Animals	CAP_{IT} BaP 5	
	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.)	877 ± 107	967 ± 85
Metabolite Pattern (% ± S.E.)[w]		
9,10-dihydrodiol	27.5 ± 4.6	30.1 ± 3.8
4,5-dihydrodiol	2.8 ± 0.2	0.8 ± 0.5
7,8-dihydrodiol	1.8 ± 0.3	16.0 ± 3.6
4,5-quinone	1.3 ± 0.0	
3,6-,1,6-6,12-quinone	5.3 ± 0.5	5.2 ± 1.2
4,5-epoxide[y]	2.5 ± 1.1	
9 & 6-OH	2.5 ± 0.4	3.9 ± 0.4
3 & 7-OH	2.4 ± 0.2	
nonextractable	43.8 ± 5.5	43.9 ± 6.0
S metabolite	9.6 ± 1.1	

[w] Metabolite pattern value expressed as % of appearance of all metabolites in blood at 60 minutes ± standard error.

[y] ^{14}C counts appear under this peak.

TABLE 24.

COMPARISON OF HPLC AND tlc DATA

Pretreatment	CAP_{IT}	
IPL	BaP	
No. of Animals	5	
	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.)	877 ± 107	967 ± 85
Metabolite Pattern (% ± S.E.)[w]		
9,10-dihydrodiol	27.5 ± 4.6	30.1 ± 3.8
4,5-dihydrodiol	2.8 ± 0.2	0.8 ± 0.5
7,8-dihydrodiol	1.8 ± 0.3	16.0 ± 3.6
4,5-quinone	1.3 ± 0.0	
3,6-,1,6-6,12-quinone	5.3 ± 0.5	5.2 ± 1.2
4,5-epoxide[y]	2.5 ± 1.1	
9 & 6-OH	2.5 ± 0.4	3.9 ± 0.4
3 & 7-OH	2.4 ± 0.2	
nonextractable	43.8 ± 5.5	43.9 ± 6.0
S metabolite	9.6 ± 1.1	

[w] Metabolite pattern value expressed as % of appearance of all metabolites in blood at 60 minutes ± standard error.

[y] ^{14}C counts appear under this peak.

TABLE 25.

COMPARISON OF HPLC AND tlc DATA

Pretreatment	$(\text{BaP} + \text{CAP})_{\text{IT}}$	
IPL	BaP	
No. of Animals	5	
	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.)	1152 ± 214	1116 ± 213
Metabolite Pattern (% ± S.E.)[w]		
9,10-dihydrodiol	26.0 ± 5.4	28.9 ± 4.6
4,5-dihydrodiol	2.5 ± 0.5	4.4 ± 1.9
7,8-dihydrodiol	3.1 ± 0.4	11.3 ± 3.2
4,5-quinone	1.3 ± 0.3	
3,6-,1,6-,6,12-quinone	5.1 ± 0.9	6.3 ± 1.5
4,5-epoxide[y]	3.6 ± 0.6	
9 & 6-OH	3.1 ± 0.4	6.0 ± 1.1
3 & 7-OH	3.4 ± 0.6	
nonextractable	43.5 ± 6.4	43.0 ± 6.1
S metabolite	8.3 ± 2.0	

[w] Metabolite pattern value expressed as % of appearance of all metabolites in blood at 60 minutes ± standard error.

[y] ^{14}C counts appear under this peak.

TABLE 26.

INFLUENCE OF PARTICULATE PRETREATMENT
% OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE
AT 180 MINUTES ± S.E.

Pretreatment	-	CAP_{IT}	$(CAP + BAP)_{IT}$
IPL	BaP	BaP	BaP
No. of Animals	3	1	2
% Unmetabolized BaP	65.3 ± 4.3	20.7	21.3 ± 2.2
% of Total Compound as Metabolite in Tissue			
Blood	15.5 ± 5.2	24.0	31.9 ± 3.5
TB	3.7 ± 1.2	8.3	3.5 ± 2.0
MAC	0.3 ± 0.1	5.3	0.9 ± 0.2
WO	3.3 ± 2.3	2.1	11.8 ± 4.5
Lung	12.0 ± 1.9	39.6	30.7 ± 0.2
% of Total Compound as BaP in Tissue			
Blood	12.7 ± 1.4	3.6	1.6 ± 0.6
TB	10.4 ± 2.7	2.4	2.1 ± 0.1
MAC	8.7 ± 7.4	1.5	2.3 ± 0.0
WO	2.5 ± 1.1	0.6	2.4 ± 0.3
Lung	31.0 ± 3.7	12.5	12.9 ± 0.8

TABLE 27.

% DISTRIBUTION PATTERN OF BaP + METABOLITES IN EACH TISSUE

LUNG[x]

Pretreatment	None	CAP_{IT}	$(CAP+BaP)_{IT}$
IPL	BaP	BaP	BaP
7,8-dihydrodiol	1.2±0.1	7.7	7.2±2.2
9,10-dihydrodiol	2.9±1.5	20.5	7.8±2.9
4,5-dihydrodiol	0.9±0.4	0.8	1.7±0.7
monohydroxylated	10.1±0.6	8.3	5.7±1.0
diones	1.1±0.3	10.2	4.0±0.3
nonextractable	5.3±0.9	18.3	32.0±5.8
BaP	78.5±2.7	34.2	41.5±1.3

[x] Based on % total activity at 180 minutes in each tissue ± S.E.
Number of animals 3,1 & 2 respectively.

(continued)

TABLE 27.

(continued)

180 BLOOD[x]

Pretreatment	None	CAP_{IT}	$(CAP+BaP)_{IT}$
IPL	BaP	BaP	BaP
7,8-dihydrodiol	2.8±0.5	19.4	7.0±0.7
9,10-dihydrodiol	6.5±3.8	44.1	20.8±6.7
4,5-dihydrodiol	1.2±0.7	1.5	4.4±3.9
monohydroxylated	2.5±0.9	1.9	3.0±0.0
diones	1.3±0.1	4.4	1.6±0.1
nonextractable	18.6±2.5	9.1	55.1±15.1
BaP	67.0±6.5	19.8	8.0±3.7

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Number of animals 3,1 & 2 respectively.

(continued)

TABLE 27.

(continued)

MACROPHAGE[x]

Pretreatment	None	CAP_{IT}	$(CAP+BaP)_{IT}$
IPL	BaP	BaP	BaP
7,8-dihydrodiol	1.4±0.4	35.6	3.6±1.5
9,10-dihydrodiol	0.9±0.2	14.1	3.0±1.3
4,5-dihydrodiol	0.2±0.2	1.8	0.6±0.5
monohydroxylated	0.9±0.3	2.3	3.7±1.1
diones	0.5±0.2	10.7	3.7±0.6
nonextractable	1.9±1.0	3.5	5.4±0.4
BaP	94.2±2.2	32.1	81.9±4.8

[x] Based on % total activity at 180 minutes in each tissue ± S.E. Number of animals 3,1 & 2 respectively.

(continued)

TABLE 27.

(continued)

WASHOUT[x]

Pretreatment	None	CAP_{IT}	$(CAP+BaP)_{IT}$
IPL	BaP	BaP	BaP
7,8-dihydrodiol	5.0±1.8	11.2	10.4±3.0
9,10-dihydrodiol	10.8±8.3	13.7	16.9±3.7
4,5-dihydrodiol	0.8±0.6	0.5	1.3±0.7
monohydroxylated	2.7±2.0	1.2	4.4±0.4
diones	2.1±1.3	8.2	11.0±6.5
nonextractable	17.5±6.6	30.8	27.4±3.2
BaP	61.0-7.7	34.3	28.6-10.2

[x] Based on % total activity at 180 minutes in each tissue ± S.E. Number of animals 3,1 & 2 respectively.

(continued)

TABLE 27.

(continued)

TRACHEA BRONCHI[x]

Pretreatment	None	CAP_{IT}	$(CAP+BaP)_{IT}$
IPL	BaP	BaP	BaP
7,8-dihydrodiol	0.8±0.2	4.1	3.9±0.1
9,10-dihydrodiol	2.2±1.2	4.0	4.0±1.5
4,5-dihydrodiol	0.9±0.5	0.0	1.0±0.4
monohydroxylated	3.4±1.8	6.5	20.4±16.6
diones	2.1±1.4	38.1	2.9±2.9
nonextractable	12.5±4.1	15.2	13.7±1.7
BaP	79.4±7.4	32.1	54.1±13.8

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Number of animals 3,1 & 2 respectively.

d. Influence of Crude Air Particulate on BaP Metabolism

1) Rate of Metabolism

CAP pretreatment appears to increase enzyme activity as indicated by the rate of metabolism (Table 28). The CAP administered concurrently with BaP on IPL, on the other hand, appears to influence metabolism in the same manner as described previously, i.e., increased rate of action of macrophages or the slow physical release of BaP. The distribution indicates that CAP pretreatment increases the amount of nonextractable and the 7,8- and 9,10-diol formation versus the control. On the other hand, CAP with BaP on the IPL causes a significant decrease in the 9,10-diol and an increase in the diones and monohydroxylated compounds. This could reflect a partial decrease in epoxide hydrase activity.

These data suggest that CAP is affecting BaP metabolism by two different mechanisms: One mechanism appears to be a long-term effect, i.e., increasing total metabolic activity, and the other is a short-term effect with a decrease in total metabolic activity which overrides the effects of pretreatment.

The HPLC supports the tlc data (Table 29). It should be noted the 7,8-dihydrodiol on tlc, 23.6%, actually consists of the 7,8-dihydrodiol plus the S metabolite (24.5%). All the other values of HPLC agree extremely well with tlc (25,55).

2) Distribution of BaP and Its Metabolites in Tissues at 180 Minutes

CAP given as a pretreatment (based on one experiment at 180 minutes) to the whole animal increases the amount of metabolite found in tissues at 180 minutes (Table 30 & 31). There is a corresponding relative increase of metabolite found in the blood and lung and a relative decrease in BaP. This is reflected in an increase in the 9,10- and 7,8-diols and a decrease in the BaP content in both tissues. The nonextractables increase in the lung and decrease in the blood compared to the control. There are some small relative increases of metabolite in the trachea bronchi and macrophage and corresponding decreases of BaP in these tissues, while there are slight relative decreases in metabolite and BaP in washout compared to the control. These data are consistent with significant increases of 7,8- and 9,10-diols and quinones in both the macrophage and washout, increases of the diones in the trachea bronchi and a significant increase of the nonextractables in the washout. These data suggest that CAP given as a pretreatment acts as an enzyme inducer; an increase in the rate of metabolism of BaP is reflected in an increase of diol formation in the tissues of the lung and smaller increases in the nonextractables, diones, and phenols.

When CAP is added to the whole animal followed by BaP adsorbed on CAP on the IPL, the relative amount of metabolism decreases compared to its control at 180 minutes (Table 30,31). There is a decrease in relative amount of metabolite in the blood and lung while there is an increase in BaP in the lung. There is a corresponding increase in nonextractables in the blood with decreases of 9,10-diol for blood and lung. A significant relative increase of BaP in the macrophage is observed for BaP_{IT} followed by BaP + CAP on IPL compared to its control. There are no such changes observed for washout or trachea bronchi. These latter observations are consistent with a decrease in the 7,8- and 9,10-diols and diones in the macrophage while the washout shows an increase in the 7,8-dihydrodiol and nonextractables and the trachea bronchi shows a significant decrease in diones.

These data suggest that CAP_{IT} followed by BaP adsorbed on CAP significantly changes the relative amounts of metabolite and BaP in tissues at 180 minutes compared to CAP_{IT} followed by BaP on IPL. The metabolite distribution has also changed in that there is a decrease in the diol formation and an increase in the nonextractables which suggests that BaP and CAP on the IPL tends to effect our type of pretreatment.

Summary

CAP acts as an enzyme inducer to increase the rate of metabolism of BaP. BaP adsorbed on CAP on the IPL on the other hand, appears to inhibit the action of CAP_{IT} pretreatment. Overall, these observations are reflected in decreases in diol formation and increases in BaP content. The major increase of BAP is in the macrophage which is consistent with decrease in metabolism; the BaP adsorbed on CAP administered to the IPL causes an increase in the rate of action of macrophage.

TABLE 28.

INFLUENCE OF CRUDE AIR PARTICULATE ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	-	CAP$_{IT}$[z]	CAP$_{IT}$[z]
IPL	BaP	BaP	BaP + CAP[x]
No. of Animals	9	5	5
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	256 ± 37[a]	830 ± 100	143 ± 29[a]
Metabolic pattern in blood (% ± S.E.)[w]			
7,8-dihydrodiol	6.6 ± 0.9[b]	18.2 ± 5.6	23.6 ± 7.6
9,10-dihydrodiol	15.4 ± 4.0[b]	32.6 ± 4.3	17.0 ± 7.2[c]
4,5-dihydrodiol	3.3 ± 0.6[b]	0.9 ± 0.5	1.9 ± 0.7
monohydroxylated	9.7 ± 1.1[a]	3.4 ± 0.6	6.5 ± 2.3
diones	10.6 ± 1.8[c]	5.5 ± 1.6	10.4 ± 2.3
nonextractable	54.4 ± 5.4	39.4 ± 8.0	40.6 ± 6.6

Column 1 compared to Column 2 - Column 3 compared to Column 2

[w] Metabolite pattern values expressed as percent of total rate of appearance of metabolite in blood ± standard error.

[x] 1 mg/kg

[z] 10 mg/kg, once/wk x 5

a. P = 0.01 b. P = 0.05 c. P = 0.10
(by Student-Newman-Keuls Test)

All metabolites separated by tlc.

TABLE 29.

COMPARISON OF HPLC AND tlc DATA

Pretreatment	CAP_{IT}	
IPL	BaP + CAP	
No. of Animals	5	
	HPLC	tlc
Appearance of all metabolites in blood at 60 minutes (ng/g lung ± S.E.	150 ± 25	144 ± 31
Metabolite pattern (% ± S.E.)[w]		
9,10-dihydrodiol	13.9 ± 5.0	16.3 ± 6.0
4,5-dihydrodiol	3.7 ± 1.4	1.6 ± 0.4
7,8-dihydrodiol	2.3 ± 0.4	23.6 ± 7.8
4,5-quinone	3.8 ± 0.9	
3,6-,1,6-,6,12-quinone	3.6 ± 0.9	10.0 ± 2.0
4,5-epoxide[y]	2.4 ± 0.5	
9 & 6-OH	3.1 ± 0.9	5.1 ± 1.7
3 & 7-OH	4.1 ± 0.8	
nonextractable	40.9 ± 5.9	43.4 ± 6.7
S metabolite	22.2 ± 5.9	

[w] Metabolite pattern value expressed as % of appearance of all metabolites in blood at 60 minutes ± standard error.

[y] ^{14}C counts appear under this peak.

TABLE 30.

INFLUENCE OF PARTICULATE ON BaP METABOLISM ON IPL % OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE AT 180 MINUTES ± S.E.

Pretreatment IPL No. of Animals	- BaP 3	CAP_{IT} BaP 1	CAP_{IT} BaP+CAP 3
% Unmetabolized BaP	65.3 ± 4.3	20.7	52.6 ± 11.2
% of Total Compound as Metabolite in Tissue			
Blood	15.5 ± 5.2	24.0	13.8 ± 2.7
TB	3.7 ± 1.2	8.3	4.3 ± 1.4
MAC	0.3 ± 0.1	5.3	4.9 ± 0.7
WO	3.3 ± 2.3	2.1	3.9 ± 0.6
Lung	12.0 ± 1.9	39.6	20.5 ± 3.6
% of Total Compound as BaP in Tissue			
Blood	12.7 ± 1.4	3.6	1.4 ± 0.5
TB	10.5 ± 2.7	2.4	4.3 ± 1.6
MAC	8.7 ± 7.4	1.5	29.5 ± 6.5
WO	2.5 ± 1.1	0.6	0.4 ± 0.1
Lung	30.9 ± 3.7	12.5	17.1 ± 3.3

TABLE 31.

% DISTRIBUTION PATTERN OF BaP + METABOLITES IN EACH TISSUE

LUNG[x]

Pretreatment	None	CAP	CAP_{IT}
IPL	BaP	BaP	BaP + CAP
7,8-dihydrodiol	1.2±0.1	7.7	5.9±0.6
9,10-dihydrodiol	2.9±1.5	20.5	6.0±2.0
4,5-dihydrodiol	0.9±0.4	0.8	2.4±0.8
monohydroxylated	10.1±0.6	8.3	4.4±1.5
diones	1.1±0.3	10.2	2.1±1.3
nonextractable	5.3±0.9	18.3	13.6±6.0
BaP	78.5±2.7	34.2	66.6±4.2

[x] Based on % total activity at 180 minutes in each tissue ± S.E. Number of animals per experiment 3,1 & 3 respectively.

(continued)

TABLE 31.

(continued)

180 BLOOD[x]

Pretreatment	None	CAP	CAP_{IT}
IPL	BaP	BaP	BaP + CAP
7,8-dihydrodiol	2.8±0.5	19.4	8.4±2.3
9,10-dihydrodiol	6.5±3.8	44.1	14.3±9.8
4,5-dihydrodiol	1.2±0.7	1.5	1.7±0.5
monohydroxylated	2.5±0.9	1.9	8.9±4.3
diones	1.3±0.1	4.4	4.5±1.4
nonextractable	18.6±2.5	9.1	40.5±17.9
BaP	67.0±6.5	19.8	21.6±8.5

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Number of animals per experiment 3,1 & 3 respectively.

(continued)

TABLE 31.

(continued)

MACROPHAGE[x]

Pretreatment	None	CAP	CAP_{IT}
IPL	BaP	BaP	BaP + CAP
7,8-dihydrodiol	1.4±0.4	35.6	2.2±0.6
9,10-dihydrodiol	0.9±0.2	14.1	0.4±0.1
4,5-dihydrodiol	0.2±0.2	1.8	0.0±0.0
monohydroxylated	0.9±0.3	2.3	0.4±0.2
diones	0.5±0.2	10.7	2.2±1.1
nonextractable	1.9±1.0	3.5	2.0±0.6
BaP	94.2±2.2	32.1	92.7±2.5

[x] Based on % total activity at 180 minutes in each tissue ± S.E. Number of animals per experiment 3,1 & 3 respectively.

(continued)

TABLE 31.

(continued)

WASHOUT[x]

Pretreatment	None	CAP	CAP_{IT}
IPL	BaP	BaP	BaP + CAP
7,8-dihydrodiol	5.0±1.8	11.2	23.4±7.0
9,10-dihydrodiol	10.8±8.3	13.7	12.1±4.6
4,5-dihydrodiol	0.8±0.6	0.5	1.3±0.2
monohydroxylated	2.7±2.0	1.2	2.4±0.5
diones	2.1±1.3	8.2	4.1±0.5
nonextractable	17.5±6.6	30.8	37.4±9.2
BaP	61.0±7.7	34.3	19.4±1.8

[x] Based on % total activity at 180 minutes in each tissue ± S.E. Number of animals per experiment 3,1 & 3 respectively.

(continued)

TABLE 31.

(continued)

TRACHEA BRONCHI[x]

Pretreatment	None	CAP	CAP_{IT}
IPL	BaP	BaP	BaP + CAP
7,8-dihydrodiol	0.8±0.2	4.1	6.0±1.4
9,10-dihydrodiol	2.2±1.2	4.0	6.1±3.3
4,5-dihydrodiol	0.9±0.5	0.0	0.6±0.1
monohydroxylated	3.4±1.8	6.5	4.2±2.3
diones	2.1±1.4	38.1	3.3±2.3
nonextractable	12.5±4.1	15.2	11.9±2.6
BaP	79.4±7.4	32.1	67.9±4.3

[x] Based on % total activity at 180 minutes in each tissue ± S.E. Number of animals per experiment 3,1 & 3 respectively.

3. Effects of SO_2

a. Influence of SO_2 Pretreatment on BaP Metabolism

1) Rate of Metabolism

SO_2 pretreatment significantly increases the rate of metabolism of BaP when compared to its control (Table 32a & b); but this increase is significantly smaller when compared to BaP pretreatment, a well characterized MFO (mixed function oxidase) enzyme inducer. SO_2, therefore, acts as a biochemical agent which causes biochemical changes in the lung due to irritation of SO_2 (15,26,42).

The distribution data do not show any marked changes when SO_2 pretreatment is compared to its control. There is a slight increase in the nonextractables and a slight decrease in monohydroxylated compounds and a larger decrease in dione formation. However, BaP pretreatment, on the other hand, shows a marked increase in the 9,10-dihydrodiol and a smaller decrease in monohydroxylated and diones when compared to SO_2 pretreatment or its control. This indicates that the SO_2 pretreatment does not have a marked effect on the metabolic pathways, i.e. epoxidation, hydroxylation and/or isomerization and conjugation (37,39,72,73).

In order to validate the tlc data, the HPLC data for SO_2 pretreatment is compared to the tlc data (Table 33) which not only supports but complements the data in Table 32a. The rates of formation, the 4,5-dihydrodiols, the nonextractables and the hydroxylated metabolites are very comparable. There are some differences, however, which are not totally unexpected considering the differences in the techniques. There is an increase in the quinones and a small decrease in 9,10-dihydrodiol for the HPLC data when compared to the tlc data. The 7,9-dihydrodiol and the S metabolite (ninhydrin positive and fluorescent which appears after BaP), on HPLC, chromatograph together on tlc and show some differences.

The HPLC data for the control are compared to the tlc data (Table 34) using four samples. These data show essentially no differences between the tlc and HPLC methods (25,55).

2) Distribution of BaP and Its Metabolites in Tissue at 180 Minutes

At 180 minutes, more BaP has been metabolized in the IPL under BaP_{IT} pretreatment conditions compared to SO_2 or no pretreatment situation (Table 35). These results are consistent with rate of appearance of metabolites in blood (Table 32a). When SO_2 is given a pretreatment there is a significant increase in the amount of metabolite in lung and a decrease in the amount of BaP in trachea bronchi and macrophage (Table 36, 37). This is reflected in an increase in the nonextractables and a decrease in the monohydroxylated compounds in the lung. The washout data shows a decrease in the 9,10-diol and nonextractables, trachea bronchi show an increase in the nonextractables and blood and macrophage do not show any significant changes in metabolite pattern.

TABLE 32a

INFLUENCE OF SO_2 PRETREATMENT ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment IPL No. of Animals	None BaP 9	$(SO_2)_{IT}$[a] BaP 5	BaP_{IT}[b] BaP 5
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	256±38	514±95[d]	1290±114[d,e]
Metabolic pattern in blood (% ± S.E.)[c]			
7,8-dihydrodiol	6.6±0.9	6.0±1.0	5.4±1.8
9,10-dihydrodiol	15.4±4.0	15.3±6.6	32.8±3.8[f]
4,5-dihydrodiol	3.3±0.6	1.2±0.4[f]	1.8±0.5[f]
Monohydroxylated	9.7±1.1	7.9±1.7	5.9±1.0
Diones	10.6±1.8	3.8±0.8[e]	2.8±0.9[e]
Nonextractables	54.4±5.4	65.7±0.4	51.8±2.8

All metabolites separated by tlc. All columns compared to each other.

[a] 1.6 - 0.4 ppm, 45 minutes.

[b] 10 mg/kg once/week x 5.

[c] Metabolite pattern values expressed as percent of total rate of appearance of metabolite in blood ± S.E.

[d] $P = 0.05$

[e] $P = 0.01$

[f] $P = 0.1$

(Statistics performed by Student-Newman-Keuls Test)

TABLE 32b

INFLUENCE OF SO$_2$ PRETREATMENT ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment IPL No. of Animals	None BaP 4	(SO$_2$)[a] BaP 5
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	334±40	547±69[c]
Metabolic pattern in blood (% ± S.E.)[b]		
9,10-dihydrodiol	20.1±6.4	10.4±4.3
4,5-dihydrodiol	2.8±0.4	2.5±0.6
7,8-dihydrodiol	2.7±0.6	4.0±0.6
4,5-quinone	1.4±0.6	2.3±0.3
1,6-6,12-3,6-quinone	2.3±0.6	5.0±1.8
4,5-epoxide[d]	2.3±1.0	2.5±0.5
9,6-OH	2.1±0.9	2.6±0.7
7-OH	2.6±0.3[e]	2.2±0.6[f]
3-OH		1.6±0.5
S Metabolite	9.4±3.8	7.7±2.4
Nonextractable	54.3±7.7	59.0±4.7

All metabolites separated by HPLC. Columns compared to each other.

[a] 1.6 ± 0.4 ppm, 45 minutes.

[b] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[c] P = 0.5 (Student-Newman-Keuls Test)

[d] ^{14}C counts appear under this peak.

[e] 7-OH and 3-OH collected together.

[f] 7-OH & 3-OH combined 1.9 ± 0.4.

TABLE 33.

COMPARISON HPLC AND tlc DATA

Pretreatment	SO_2
IPL	BaP
No. of Animals	5

	HPLC	tlc
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	547±69	514±95
Metabolic pattern in blood (% ± S.E.)[a]		
9,10-dihydrodiol	10.4±4.3	15.3±6.6
4,5-dihydrodiol	2.5±0.6	1.2±0.3
7,8-dihydrodiol	4.0±0.6	6.0±1.0
4,5-quinone	2.3±0.3	3.8±0.8
1,6-6,12-3,6-quinone	5.0±1.8	
4,5-epoxide[b]	2.5±0.5	
9,6-OH	2.6±0.7	7.9±1.6
7-OH	2.2±0.6	
3-OH	1.6±0.5	
S Metabolite	7.7±2.4	
Nonextractable	59.0±4.7	65.7±0.4

[a] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[b] ^{14}C counts appear under peak.

TABLE 34.

COMPARISON OF HPLC AND tlc DATA

Pretreatment	-	
IPL	BaP	
No. of Animals	4	
	HPLC	tlc
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	334±40	364±45
Metabolic pattern in blood (% ± S.E.)[a]		
9,10-dihydrodiol	20.1±6.4	18.3±7.5
4,5-dihydrodiol	2.8±0.4	2.2±1.0
7,8-dihydrodiol	2.7±0.6	10.3±2.7
4,5-quinone	1.4±0.6	3.6±0.8
1,6-6,12-3,6-quinone	2.3±0.6	
4,5-epoxide[b]	2.3±1.0	
9,6-OH	2.1±0.9	6.3±2.7
7-OH	2.6±0.3[c]	
3-OH		
S Metabolite	9.4±3.8	
Nonextractable	54.3±7.7	59.2±9.9

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[a] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[b] ^{14}C counts appear under peak.

[c] 7-OH and 3-OH collected together.

TABLE 35.

INFLUENCE OF SO_2 PRETREATMENT ON BaP METABOLISM* % OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE AT 180 MINUTES ± S.E.

Pretreatment	-	SO_2*	BaP_{IT}
IPL	BaP	BaP	BaP
No. of Animals	3	3	3
% Unmetabolized BaP	65.3 ± 4.3	46.9 ± 5.2	21.9 ± 3.1
% of Total Compound as Metabolite in Tissue			
Blood	15.5 ± 5.2	16.2 ± 3.0	41.5 ± 7.5
TB	3.7 ± 1.2	6.2 ± 1.4	1.3 ± 0.5
MAC	0.3 ± 0.1	0.5 ± 0.1	0.9 ± 0.2
WO	3.3 ± 2.3	2.9 ± 0.2	6.3 ± 0.5
Lung	12.0 ± 1.9	26.9 ± 6.0	28.0 ± 1.4
% of Total Compound as BaP in Tissue			
Blood	12.7 ± 1.4	10.5 ± 3.3	1.9 ± 0.2
TB	10.4 ± 2.7	2.9 ± 1.4	1.5 ± 0.5
MAC	8.7 ± 7.4	2.0 ± 0.4	4.7 ± 0.6
WO	2.5 ± 1.1	3.0 ± 0.3	2.4 ± 0.6
Lung	30.9 ± 3.6	28.6 ± 3.4	11.5 ± 3.0

*tlc data

TABLE 36.

% DISTRIBUTION PATTERN OF BaP + METABOLITES IN EACH TISSUE[t]

LUNG[x]			
Pretreatment	None	SO_2*	BaP_{IT}
IPL	BaP	BaP	BaP
7,8-dihydrodiol	1.2±0.1	3.8±0.6	5.4±0.7
9,10-dihydrodiol	2.9±1.5	2.2±0.4	2.7±0.6
4,5-dihydrodiol	0.9±0.4	0.2±0.1	0.9±0.3
monohydroxylated	10.1±0.6	2.2±0.2	3.5±1.4
diones	1.1±0.3	2.4±0.3	3.7±0.7
nonextractable	5.3±0.9	10.0±0.7	43.9±4.6
BaP	78.5±2.7	79.2±1.9	39.8±7.4

*SO_2 = 120 minutes
BaP

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

[t] There are 3 animals each for controls, SO_2, and BaP_{IT} - tlc data.

(continued)

TABLE 36.

(continued)

180 BLOOD[x]

Pretreatment	None	SO_2*	BaP_{IT}
IPL	BaP	BaP	BaP
7,8-dihydrodiol	2.8±0.5	1.9±0.3	6.4±1.4
9,10-dihydrodiol	6.5±3.8	2.4±0.4	17.4±2.5
4,5-dihydrodiol	1.2±0.7	0.3±0.1	1.9±1.2
monohydroxylated	2.5±0.9	2.2±0.3	3.3±0.6
diones	1.3±0.1	3.3±0.9	1.9±0.4
nonextractable	18.6±2.5	20.8±2.6	61.5±2.5
BaP	67.0±6.5	69.1±2.8	7.6±1.4

*SO_2 = 120 minutes
BaP

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

[t] There are 3 animals each for controls, SO_2, and BaP_{IT} - tlc data.

(continued)

TABLE 36.

(continued)

MACROPHAGE[x]			
Pretreatment	None	SO_2*	BaP_{IT}
IPL	BaP	BaP	BaP
7,8-dihydrodiol	1.4±0.4	1.2±0.2	1.4±0.3
9,10-dihydrodiol	0.9±0.2	0.7±0.1	0.9±0.2
4,5-dihydrodiol	0.2±0.2	0.1±0.0	0.1±0.0
monohydroxylated	0.9±0.3	2.8±0.6	1.3±0.6
diones	0.5±0.2	0.8±0.2	2.4±1.0
nonextractable	1.9±1.0	1.5±0.2	3.5±0.9
BaP	94.2±2.2	93.0±1.1	90.3±2.5

*SO_2 = 120 minutes
BaP

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

[t] There are 3 animals each for controls, SO_2, and BaP_{IT} - tlc data.

(continued)

TABLE 36.

(continued)

WASHOUT[x]

Pretreatment	None	SO_2*	BaP_{IT}
IPL	BaP	BaP	BaP
7,8-dihydrodiol	5.0±1.8	2.0±0.5	5.5±1.8
9,10-dihydrodiol	10.8±8.3	2.3±0.7	7.1±1.2
4,5-dihydrodiol	0.8±0.6	0.5±0.1	0.6±0.4
monohydroxylated	2.7±2.0	5.5±0.3	3.8±1.1
diones	2.1±1.3	3.3±1.9	3.8±1.3
nonextractable	17.5±6.6	6.9±2.3	39.6±4.6
BaP	61.0±7.7	78.4±2.9	39.6±2.2

*SO_2 = 120 minutes
BaP

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

[t] There are 3 animals each for controls, SO_2, and BaP_{IT} - tlc data.

(continued)

TABLE 36.

(continued)

TRACHEA BRONCHI[x]

Pretreatment	None	SO_2*	BaP_{IT}
IPL	BaP	BaP	BaP
7,8-dihydrodiol	0.8±0.2	3.4±1.5	4.4±0.7
9,10-dihydrodiol	2.2±1.2	1.6±0.3	3.4±0.8
4,5-dihydrodiol	0.9±0.5	0.2±0.1	0.5±0.2
monohydroxylated	3.4±1.8	1.7±0.2	1.4±0.7
diones	2.1±1.4	5.7±3.2	3.2±2.6
nonextractable	12.5±4.1	27.0±6.2	24.8±11.5
BaP	79.4±7.4	60.4±6.1	62.2±10.1

*SO_2 = 120 minutes
BaP

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

[t] There are 3 animals each for controls, SO_2, and BaP_{IT} - tlc data.

BaP_{IT} pretreatment shows a large increase in metabolite in the blood, a corresponding decrease of BaP in the blood and lung when compared to SO_2 and/or control. There is a comparable increase of metabolite in the lung as in the case for SO_2 pretreatment. This is reflected in an increase in the nonextractables in the lung, washout, and blood, and an increase in 9,10-diol in the blood as shown in Table 32a for BaP_{IT} pretreatment compared to SO_2 pretreatment and/or control. An increase in the nonextractables in the trachea bronchi is present for both SO_2 and BaP pretreatment.

These data suggest that on a percentage basis more metabolite is found in the IPL, i.e. lung, washout, and blood, after BaP_{IT} pretreatments than SO_2 pretreatments and controls at 180 minutes. This is reflected in a general increase in the nonextractables in lung, washout and blood.

Summary

These data indicate that initially SO_2 pretreatment increases the rate of metabolism compared to control but does not significantly change the metabolic pattern. The SO_2 pretreatment data, however, are very different from that obtained for an enzyme inducer which affects not only the rate but also the pattern. This difference in rate between BaP_{IT} and SO_2 and control is reflected in a large increase in the 9,10-diol and a slight decrease in the nonextractables.

After 180 minutes, similar results are obtained in that the amount of BaP unmetabolized in each case is consistent with rate of metabolism. In general, there are some small increases in the amount of nonextractables for SO_2 pretreatments compared to control while there are some rather large increases in the nonextractables from BaP_{IT} pretreatments compared to SO_2 and control. These data again suggest that SO_2 pretreatments show an increase in the rate of metabolism but does not significantly affect the pattern. SO_2, therefore, does not appear to affect BaP metabolism by enzymatic induction.

b. Influence of SO_2 Administered to IPL on BaP Metabolism

1) Rate of Metabolism

SO_2 administered to the IPL significantly increases the rate of metabolism of BaP compared to its control (Tables 37,40). This suggests, in the same manner described previously, that SO_2 acts as a biochemical agent which causes an increase in the enzyme activity or as a physical agent which causes biochemical changes in the lung (26,42). However, CAP administered concurrently with BaP and SO_2 to the IPL, appears to significantly decrease the rate of metabolism of BaP when compared to its appropriate control. A factor to consider is that BaP may not be leached readily from the CAP and therefore, is not available to be metabolized.

The metabolite pattern does not show any marked changes when the SO_2 treatment is compared to its control. There are small increases in the 9,10-dihydrodiol and nonextractables and small decreases in monohydroxylated and dione formation. When CAP is administered concurrently with BaP and SO_2 to the IPL there is a decrease in the nonextractables and small increases or no change in the 9,10- and 7,8-dihydrodiols (Tables 37,40). This indicates that SO_2 administered in vitro on the IPL does not have major effects on the metabolic pattern.

In order to validate the tlc data, the HPLC data for SO_2 and BaP on the IPL, as well as SO_2, CAP and BaP on the IPL are compared to tlc (Tables 38,39). The rates, nonextractables, the monohydroxylated and 4,5- and 9,10-dihydrodiols are very comparable. The diones in Table 38 are comparable while the diones in Table 39 show an increase for HPLC data when compared to the tlc data. In both Tables 38 and 39 the 7,8-dihydrodiol from the tlc data is very comparable to the 7,8-dihydrodiol plus the S metabolite (appears after BaP on chromatogram and is fluorescent and ninhydrin positive) for HPLC data (25,55).

2) Distribution of BaP and Its Metabolites in Tissue at 180 Minutes

At 180 minutes, there is a small decrease in the (Tables 40,41) amount of unmetabolized BaP compared to control. This is consistent with small increases of metabolite in blood and lung and BaP in the blood with corresponding decreases of BaP in trachea bronchi and lung. These data are consistent with small increases in the 7,8- and 9,10-diols in lung, blood washout, macrophage, and trachea bronchi and other minor changes in monohydroxylated diones and nonextractables. Therefore, as similarly stated in Section b,1), SO with BaP on IPL does not have major effects on the metabolic pattern.

When CAP and SO_2 are administered together with BaP on the IPL, there appears to be a dramatic increase in metabolism of BaP after 180 minutes compared to SO_2 and BaP alone (Tables 40,41). There also appears a change in the rate of metabolism of BaP due to CAP plus SO_2 as seen from the results in Tables 37 and 41. Initially the BaP may not be leached readily from CAP and therefore, is not available to be metabolized. However, after a period of time, the BaP is probably leached from the CAP at a faster rate. By this time the SO_2 has had an effect on the lung such as to cause cellular injury and/or a change in normal enzyme functions. These changes result in an increase in the amount of BaP metabolized.

These observations and assumptions are reflected in large increases of metabolite in blood, trachea bronchi and lung and large decreases of BaP in blood and lung compared to SO_2 and BaP alone. These data are consistent with relative increases in diol and nonextractables in blood and washout and relative increases in diones and nonextractables in the trachea bronchi. No such changes are seen for the lung and macrophage. This last statement is not consistent with the data obtained for the lung as seen in Table 41.

All of these data suggest that CAP and SO_2 administered together with BaP on the IPL causes an increase in the rate of metabolism after an equilibrium period compared to control. This increase is reflected in more metabolite in blood, trachea bronchi, and lung at 180 minutes with most of the metabolite increase attributed to the 7,8- and/or 9,10-diol and nonextractable formation.

Summary

These data indicate that initially SO_2 on the IPL increases the rate of metabolism of BaP compared to control but does not significantly change the metabolite pattern. When CAP and SO_2 are administered to the IPL, the rate of metabolism of BaP is significantly decreased compared to its appropriate control. This could be due to the fact that BaP is not leached readily from the CAP. The CAP and SO_2 together do, however, cause small changes in the metabolic pattern compared to the control with increases in the 7,8- and 9,10-diol and a decrease in nonextractables.

At 180 minutes, the amount of unmetabolized BaP left for BaP and BaP plus SO_2 are consistent with rate of metabolism. The SO_2 does not appear to have major effects on the metabolic pattern. CAP plus SO_2 administered together with BaP, on the other hand, causes a significant increase in the rate of metabolism of BaP after an equilibration period. This can be due to the combination of leaching of BaP from CAP at a faster rate and changes in normal enzyme functions and/or cytopathological effects due to the SO_2. This increase in metabolism is attributed to the 7,8- and/or 9,10-diol and nonextractable formation. Under these conditions, therefore, SO_2 may cause an increase in the enzyme activity.

TABLE 37.

INFLUENCE OF SO_2 ADMINISTERED TO IPL ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	None	None	None
IPL	BaP	BaP+SO_2[a]	BaP+CAP+SO_2[b]
No. of Animals	9	5	7
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	256±38[d]	551±77	177±48[d]
Metabolic pattern in blood (% ± S.E.)[c]			
7,8-dihydrodiol	6.6±0.9	6.0±0.7	11.0±1.6[f]
9,10-dihydrodiol	15.4±4.0	20.0±2.1	26.7±2.9[e]
4,5-dihydrodiol	3.3±0.6	2.0±0.6	1.7±0.7
Monohydroxylated	9.7±1.1[d]	4.2±0.6	6.3±1.1
Diones	10.6±1.8	7.9±2.6	6.9±2.0
Nonextractables	54.4±5.4[e]	59.9±3.4	42.4±2.6[d]

All metabolites separated by tlc. Columns 1 & 3 compared to Column 2.

[a] 2.2 ± 0.1 ppm SO_2

[b] 10 mg/kg CAP, 2.1 ± 0.1 ppm SO_2

[c] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[d] $P = 0.01$
[e] $P = 0.1$
[f] $P = 0.05$
(Statistics performed by Student-Newman-Keuls Test)

TABLE 38.

COMPARISON HPLC AND tlc DATA

Pretreatment	None	
IPL	BaP+SO_2	
No. of Animals	5	
	HPLC	tlc
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	588±60	551±77
Metabolic pattern in blood (% ± S.E.)[a]		
9,10-dihydrodiol	18.9±2.1	20.0±2.1
4,5-dihydrodiol	2.5±0.6	2.0±0.6
7,8-dihydrodiol	2.0±0.4	6.0±0.7
4,5-quinone	2.0±0.3	7.9±2.6
1,6-,6,12-,3,6-quinones	3.3±1.1	
4,5-epoxide[b]	4.4±0.5	
9-,6-OH	2.7±0.4	4.2±0.6
7-OH	1.7±0.5	
3-OH	1.4±0.4	
S Metabolite	6.0±0.4	
Nonextractable	55.1±4.5	59.9±3.4

[a] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[b] ^{14}C counts appear under peak.

TABLE 39.

COMPARISON HPLC AND tlc DATA

Pretreatment	None	
IPL	BaP+SO_2+CAP	
No. of Animals	7	
	HPLC	tlc
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	191±53	177±47
Metabolic pattern in blood (% ± S.E.)[a]		
9,10-dihydrodiol	17.4±3.5	26.7±2.9
4,5-dihydrodiol	3.5±0.6	1.7±0.7
7,8-dihydrodiol	5.7±0.7	11.0±1.6
4,5-quinone	4.3±1.9	6.9±2.0
1,6-,6,12-,3,6-quinones	5.0±0.9	
4,5 epoxide[b]	5.9±0.6	
9-,6-OH	2.5±0.4	6.3±1.1
7-OH	3.0±0.6	
3-OH	2.8±0.8	
S Metabolite	5.9±1.2	
Nonextractable	43.9±4.0	42.4±2.6

[a] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[b] ^{14}C counts appear under peak.

TABLE 40.

INFLUENCE OF SO_2 ADMINISTERED TO IPL ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	None	None	None
IPL	BaP	$BaP+SO_2$[a]	$BaP+CAP+SO_2$[b]
No. of Animals	4	5	7
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	334±40[e]	588±60	191±53[d]
Metabolic pattern in blood (% ± S.E.)[c]			
9,10-dihydrodiol	20.1±6.4	18.9±2.1	17.4±3.5
4,5-dihydrodiol	2.8±0.4	2.5±0.6	3.5±0.6
7,8-dihydrodiol	2.7±0.6	2.0±0.4	5.7±0.7[d]
4,5-quinone	1.4±0.6	2.0±0.3	4.3±1.9
1,6-6,12-3,6-quinone	2.3±0.6	3.3±1.1	5.1±0.9
4,5-epoxide[g]	2.3±1.0[f]	4.4±0.5	5.9±0.6
9,6-OH	2.1±0.9	2.7±0.4	2.5±0.4
7-OH	2.6±0.3[f,h]	1.7±0.5[i]	3.0±0.6
3-OH		1.4±0.4[i]	2.8±0.8
S Metabolite	9.4±3.8	6.0±0.4	5.9±1.1
Nonextractable	54.3±7.7	55.1±4.5	43.9±4.0[f]

All metabolites separated by HPLC. Columns 1 & 3 compared to Column 2.

[a] 2.2 ± 0.1 ppm SO_2

[b] 10 mg/kg CAP, 2.1 ± 0.1 ppm SO_2

[c] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[d] P = 0.01

[e] P = 0.05

[f] P = 0.1 (Student-Newman-Keuls Test)

[g] ^{14}C counts appear under this peak.

[h] 7-OH and 3-OH collected together.

[i] 7-OH and 3-OH combined 1.6 ± 0.3

TABLE 41.

INFLUENCE OF SO_2 ADMINISTERED TO IPL ON BaP METABOLISM* % OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE AT 180 MINUTES ± S.E.

Pretreatment	-	-	-
IPL	BaP	BaP+SO_2	BaP+SO_2+CAP
No. of Animals	3	3	2
% of Unmetabolized BaP	65.3 ± 4.3	55.2 ± 4.7	18.5 ± 8.6
% of Total Compound as Metabolite in Tissue			
Blood	15.5 ± 5.2	17.4 ± 2.4	26.7 ± 11.4
TB	3.7 ± 1.2	3.3 ± 1.9	12.6 ± 7.9
MAC	0.3 ± 0.1	0.9 ± 0.1	2.9 ± 0.9
WO	3.3 ± 2.3	3.8 ± 0.6	4.7 ± 0.4
Lung	12.0 ± 1.9	19.4 ± 4.8	34.5 ± 6.9
% of Total Compound as BaP in Tissue			
Blood	12.7 ± 1.4	18.8 ± 8.6	1.2 ± 0.1
TB	10.4 ± 2.7	2.4 ± 0.6	1.1 ± 0.1
MAC	8.7 ± 7.4	3.6 ± 1.1	2.2 ± 0.7
WO	2.5 ± 1.1	3.4 ± 0.8	0.2 ± 0.0
Lung	30.9 ± 3.6	26.9 ± 0.6	13.8 ± 6.3

*tlc data

TABLE 42.

% DISTRIBUTION PATTERN OF BaP + METABOLITE IN EACH TISSUE

LUNG [x]

Pretreatment	None	None	None
IPL	BaP	BaP+SO_2	BaP+SO_2+CAP
7,8-dihydrodiol	1.2±0.1	2.0±0.3	3.8±0.7
9,10-dihydrodiol	2.9±1.5	6.7±1.4	3.9±0.3
4,5-dihydrodiol	0.9±0.4	0.3±0.1	0.5±0.0
monohydroxylated	10.1±0.6	5.6±0.8	5.6±2.5
diones	1.1±0.3	1.3±0.3	3.4±0.1
nonextractable	5.3±0.9	9.7±3.4	9.6±2.2
BaP	78.5±2.7	74.3±4.8	73.2±5.5

[x] Based on % total activity at 180 minutes in each tissue ± S.E. Number of animals are 3,3,2 respectively - tlc data.

(continued)

TABLE 42.

(continued)

180 BLOOD[x]

Pretreatment	None	None	None
IPL	BaP	BaP+SO_2	BaP+SO_2+CAP
7,8-dihydrodiol	2.8±0.5	3.1±1.4	8.0±2.5
9,10-dihydrodiol	6.5±3.8	10.2±2.7	16.6±5.2
4,5-dihydrodiol	1.2±0.7	1.0±0.6	0.8±0.0
monohydroxylated	2.5±0.9	1.8±0.1	3.6±1.9
diones	1.3±0.1	3.4±1.8	5.9±2.7
nonextractable	18.6±2.5	19.4±9.4	36.5±1.2
BaP	67.0±6.5	61.1±13.7	28.6±8.4

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,3,2 respectively - tlc data.

(continued)

TABLE 42.

(continued)

MACROPHAGE[x]

Pretreatment	None	None	None
IPL	BaP	BaP+SO_2	BaP+SO_2+CAP
7,8-dihydrodiol	1.4±0.4	4.6±2.1	8.1±2.1
9,10-dihydrodiol	0.9±0.2	1.8±0.2	0.9±0.0
4,5-dihydrodiol	0.2±0.2	0.2±0.0	0.2±0.1
monohydroxylated	0.9±0.3	1.4±0.6	0.5±0.1
diones	0.5±0.2	1.6±0.5	4.2±1.9
nonextractable	1.9±1.0	1.8±0.5	1.0±0.1
BaP	94.2±2.2	88.4±2.0	85.0±0.2

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,3,2 respectively - tlc data.

(continued)

TABLE 42.

(continued)

WASHOUT[x]

Pretreatment	None	None	None
IPL	BaP	BaP+SO_2	BAP+SO_2+CAP
7,8-dihydrodiol	5.0±1.8	10.5±6.1	20.0±10.1
9,10-dihydrodiol	10.8±8.3	10.0±1.6	9.6±6.0
4,5-dihydrodiol	0.8±0.6	2.1±1.5	0.9±0.5
monohydroxylated	2.7±2.0	4.5±1.5	3.1±1.8
diones	2.1±1.3	2.3±0.8	8.4±6.5
nonextractable	17.5±6.6	7.3±1.6	33.4±3.0
BaP	61.0±7.7	63.1±5.6	24.5±1.6

[x] Based on % total activity at 180 minutes in each tissue ± S.E. Number of animals are 3,3,2 respectively - tlc data.

(continued)

TABLE 42.

(continued)

TRACHEA BRONCHI[x]

Pretreatment	None	None	None
IPL	BaP	BaP+SO_2	BaP+SO_2+CAP
7,8-dihydrodiol	0.8±0.2	1.9±0.3	2.9±0.8
9,10-dihydrodiol	2.2±1.2	4.8±1.1	4.0±0.6
4,5-dihydrodiol	0.9±0.5	0.6±0.3	0.3±0.1
monohydroxylated	3.4±1.8	7.9±3.9	4.1±2.7
diones	2.1±1.4	10.4±9.2	17.8±8.8
nonextractable	12.5±4.1	11.2±3.4	24.2±7.0
BaP	79.4±7.4	63.3±15.1	46.7±18.4

[x] Based on % total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,3,2 respectively - tlc data.

c. Influence of SO_2 and CAP Administration to IPL on BaP Metabolism

1) Rate of Metabolism

CAP on the IPL acts to slightly inhibit the rate of metabolism of BaP (Tables 43,46). SO_2, added concurrently with BaP and CAP to the IPL, does not change the rate of metabolism when compared to CAP and BaP on the IPL. This may be due to either the slow leaching of BaP from CAP or an increase in phagocytic action of the macrophage which might serve to decrease the amount of BaP available for metabolism. Additionally, at these low concentrations of SO_2, the gas could very possibly be adsorbed by the particulate and is not available to affect the metabolism of BaP.

The distribution data due to the particulate indicate an increase in the 7,8- and 9,10-dihydrodiols and a decrease in the nonextractables, and the monohydroxylated and dione metabolites. It should be noted that the 7,8-dihydrodiol spot contains some (possibly glutathione) conjugation. This suggests that particulate affects the epoxide hydrase pathway (37,39,72,23) more readily than the hydroxylation and/or isomerization and some conjugation pathways. However, when the distribution data for SO_2, CAP, and BaP or the IPL are compared to BaP and CAP alone, there do not seem to be any significant differences except for the 7,8-dihydrodiol, which contains some unknown metabolite (possibly conjugate), and the 4,5-dihydrodiol. This suggests that SO_2 at these concentrations does not cause any marked changes in the metabolic pathways in the presence of BaP and CAP.

2) Distribution of BaP and Its Metabolites in Tissue at 180 Minutes

There is an increase in metabolism of BaP at 180 minutes when CAP is administered with BaP compared to the control (Tables 44,45). This would suggest that after an equilibration time, BaP is leached at a faster rate. This is consistent with a relative decrease of BaP in the blood at 180 minutes compared to control, while the lung and trachea bronchi show a relative increase in metabolite and a decrease in BaP. The macrophage and washout both show increases in the relative amounts of the BaP metabolite content. The only significant changes that are observed in their metabolite profiles are the 7,8- and 9,10-diols and the nonextractables for the washout fraction.

When CAP and SO_2 are administered together with BaP on the IPL, there appears to be an even more dramatic increase in the metabolism of BaP after 180 minutes compared to CAP and BaP alone (Table 44,45). There also appears a change in the rate of metabolism of BaP due to CAP plus SO_2 as seen from the results in Tables 43 and 44. Initially the BaP may not be leached readily from CAP and, therefore, is not available to be metabolized. However, after a period of time, the BaP is probably leached from the CAP at a faster rate. By this time, the SO_2 has been an effect on the lung such as to cause cellular injury and/or a change in normal enzyme functions. These changes result in an increase in the amount of BaP metabolized.

These observations are reflected in a large increase of metabolite in blood, trachea bronchi, and lung, a large decrease in metabolite in washout, a large decrease of BaP in macrophage and washout, and an increase in BaP in the lung compared to CAP and BaP alone. These data are consistent with relative increases in diol and quinone formation in blood, macrophage, washout, and trachea bronchi.

TABLE 43.

INFLUENCE OF SO_2 AND CAP ADMINISTERED TO IPL ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	None	None	None
IPL	BaP	BaP+CAP[a]	BaP+CAP+SO_2[b]
No. of Animals	9	5	7
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	256±38	156±42	177±48
Metabolic pattern in blood (% ± S.E.)[c]			
7,8-dihydrodiol	6.6±0.9[d]	19.1±4.4	11.0±1.6
9,10-dihydrodiol	15.4±4.0	28.3±7.9	26.7±2.9
4,5-dihydrodiol	3.3±0.6	3.0±1.3	1.7±0.7
Monohydroxylated	9.7±1.1[e]	5.1±1.4	6.3±1.1
Diones	10.6±1.8	5.2±2.6	6.9±2.0
Nonextractables	54.4±5.4	39.3±13.8	42.4±2.6

All metabolites separated by tlc. Columns 1 & 3 compared to Column 2.

[a] 1 mg/kg

[b] 1 mg/kg CAP, 2.1 ± 0.1 ppm SO_2

[c] Metabolite pattern values expressed as percent of total rate of appearance of metabolite in blood ± S.E.

[d] $P = 0.01$

[e] $P = 0.05$

(Statistics performed by Student-Newman-Keuls Test)

TABLE 44.

INFLUENCE OF SO_2 AND CAP ADMINISTRATION TO IPL ON BaP METABOLISM* % OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE AT 180 MINUTES ± S.E.

Pretreatment	-	-	-
IPL	BaP	BaP+CAP	BaP+CAP+SO_2
No. of Animals	3	2	2
% Unmetabolized BaP	65.3 ± 4.3	40.0 ± 26.3	18.5 ± 8.6
% of Total Compound as Metabolite in Tissue			
Blood	15.5 ± 5.2	16.6 ± 6.7	26.7 ± 11.4
TB	3.7 ± 1.2	6.2 ± 2.8	12.6 ± 7.9
MAC	0.3 ± 0.1	1.9 ± 1.3	2.9 ± 0.9
WO	3.3 ± 2.3	13.2 ± 3.3	4.7 ± 0.4
Lung	12.0 ± 1.9	22.1 ± 9.1	34.5 ± 6.9
% of Total Compound as BaP in Tissue			
Blood	12.7 ± 1.4	1.0 ± 0.8	1.2 ± 0.1
TB	10.4 ± 2.7	2.2 ± 1.0	1.1 ± 0.1
MAC	8.7 ± 7.4	17.4 ± 12.2	2.2 ± 0.7
WO	2.5 ± 1.1	10.9 ± 9.2	0.2 ± 0.0
Lung	30.9 ± 3.6	8.5 ± 2.5	13.8 ± 6.3

*tlc data

TABLE 45.

% DISTRIBUTION PATTERN OF BaP & METABOLITE IN EACH TISSUE

LUNG[x]			
Pretreatment	None	None	None
IPL	BaP	BaP+CAP	BaP+CAP+SO_2
7,8-dihydrodiol	1.2±0.1	6.1±0.5	3.8±0.7
9,10-dihydrodiol	2.9±1.5	3.2±0.2	3.9±0.3
4,5-dihydrodiol	0.9±0.4	1.3±0.2	0.5±0.0
monohydroxylated	10.1±0.6	3.4±0.7	5.6±2.5
diones	1.1±0.3	1.3±1.0	3.4±0.1
nonextractable	5.3±0.9	24.7±2.1	9.6±2.2
BaP	78.5±2.7	60.0±3.2	73.2±5.5

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,2,2 respectively - tlc data.

(continued)

TABLE 45.

(continued)

180 BLOOD[x]

Pretreatment	None	None	None
IPL	BaP	BaP+CAP	BaP+CAP+SO_2
7,8-dihydrodiol	2.8±0.5	8.0±5.8	8.0±2.5
9,10-dihydrodiol	6.5±3.8	5.6±3.4	16.6±5.2
4,5-dihydrodiol	1.2±0.7	0.9±0.6	0.8±0.0
monohydroxylated	2.5±0.9	2.2±1.8	3.6±1.9
diones	1.3±0.1	1.5±1.3	5.9±2.7
nonextractable	18.6±2.5	59.7±33.7	36.5±1.2
BaP	67.0±6.5	22.1±20.8	28.6±8.4

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,2,2 respectively - tlc data.

(continued)

TABLE 45.

(continued)

MACROPHAGE[x]

Pretreatment	None	None	None
IPL	BaP	BaP+CAP	BaP+CAP+SO_2
7,8-dihydrodiol	1.4±0.4	1.5±0.5	8.1±2.1
9,10-dihydrodiol	0.9±0.2	0.3±0.2	0.9±0.0
4,5-dihydrodiol	0.2±0.2	0.1±0.0	0.2±0.1
monohydroxylated	0.9±0.3	0.2±0.1	0.5±0.1
diones	0.5±0.2	0.2±0.1	4.2±1.9
nonextractable	1.9±1.0	1.4±0.3	1.0±0.1
BaP	94.2±2.2	96.3±1.3	85.0±0.2

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,2,2 respectively - tlc data.

(continued)

TABLE 45.

(continued)

WASHOUT[x]

Pretreatment	None	None	None
IPL	BaP	BaP+CAP	BaP+CAP+SO_2
7,8-dihydrodiol	5.0±1.8	28.4±19.6	20.0±10.1
9,10-dihydrodiol	10.8±8.3	5.3±0.5	9.6±6.0
4,5-dihydrodiol	0.8±0.6	0.9±0.3	0.9±0.5
monohydroxylated	2.7±2.0	3.2±2.1	3.1±1.8
diones	2.1±1.3	2.4±0.6	8.4±6.5
nonextractable	17.5±6.6	0±00	33.4±3.0
BaP	61.0±7.7	59.9±22.1	24.5±1.6

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,2,2 respectively - tlc data.

(continued)

TABLE 45.

(continued)

TRACHEA BRONCHI[x]

Pretreatment	None	None	None
IPL	BaP	BaP+CAP	BaP+CAP+SO_2
7,8-dihydrodiol	0.8±0.2	3.5±1.2	2.9±0.8
9,10-dihydrodiol	2.2±1.2	2.6±1.7	4.0±0.6
4,5-dihydrodiol	0.9±0.5	0.4±0.3	0.3±0.1
monohydroxylated	3.4±1.8	0.3±0.3	4.1±2.7
diones	2.1±1.4	3.5±1.5	17.8±8.8
nonextractable	12.5±4.1	26.7±16.1	24.2±7.0
BaP	79.4±7.4	63.1±21.0	46.7±18.4

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,2,2 respectively - tlc data.

TABLE 46.

INFLUENCE OF SO_2 + CAP ADMINISTERED TO IPL ON BaP METABOLISM RATE AND PATTERN OF METABOLISM IN THE BLOOD

Pretreatment	None	None
IPL	BaP	BaP+CAP+SO_2[a]
No. of Animals	4	7
Total rate of appearance of metabolites in blood (ng/hr/g lung ± S.E.)	334±40[c]	191±53
Metabolic pattern in blood (% ± S.E.)[b]		
9,10-dihydrodiol	20.1±6.4	17.4±3.5
4,5-dihydrodiol	2.8±0.4	3.5±0.5
7,8-dihydrodiol	2.7±0.6[d]	5.7±0.7
4,5-quinone	1.4±0.6	4.3±1.9
1,6-6,12-3,6-quinone	2.3±0.6[c]	5.0±0.9
4,5-epoxide[f]	2.3±1.0[e]	5.9±0.6
9,6-OH	2.1±0.9	2.5±0.4
7-OH	2.6±0.3[g]	3.0±0.6[h]
3-OH		2.8±0.8
S Metabolite	9.4±3.8	5.9±1.1
Nonextractable	54.3±7.7	43.9±4.0

All metabolites separated by HPLC.

[a] 1 mg/kg CAP, 2.1 ± 0.1 ppm SO_2.

[b] Metabolite pattern values expressed as percent of total rate of appearance of metabolites in blood ± S.E.

[c] P = 0.1

[d] P = 0.05

[e] P = 0.01 (Student-Newman-Keuls Test)

[f] ^{14}C counts appear under this peak.

[g] 7-OH and 3-OH collected together

[g] 7-OH and 3-OH combined 2.9 ± 0.5

All of these data suggest that CAP and SO_2, administered together with BaP on the IPL, cause an increase in the rate of metabolism after an equilibrium period compared to CAP and BaP alone. This is reflected in more metabolite in blood, trachea bronchi, and lung at 180 minutes.

Summary

The particulate inhibits the rate of appearance of metabolites of BaP observed in the blood. This can be explained by the relative increase of BaP and metabolite in the macrophage and washout with corresponding decrease of BaP in the lung and trachea bronchi. The BaP adsorbed particulate can be engulfed more readily by macrophages and, therefore, BaP is less biologically available for metabolism or is leached more slowly from the lung. However, at the end of 180 minutes the relative amount of BaP metabolized by CAP plus BaP is greater than the control which is consistent with an increase of metabolite in lung and trachea bronchi, as well as washout and macrophage. This then might suggest that CAP which contains a variety of PAHs and metals might induce the enzyme system slightly after a period of time for equilibration or that macrophage metabolize BaP but delay the excretion of these metabolites into the blood.

SO_2 added concurrently with BaP and CAP on the IPL does not change the rate of metabolism when compared to BaP and CAP alone. This can be due to slow leaching of BaP or phagocytic action as described previously. Additionally, at low concentrations of SO_2 the gas could very possibly be adsorbed by the particulate and not be available to affect the metabolism of BaP. After 180 minutes, however, there appears to be a dramatic increase in the metabolism of BaP. This is reflected in more metabolite in blood, trachea bronchi, and lung at 180 minutes. This can be attributed to leaching of BaP from the CAP at a faster rate and possibly to an effect on the lung by SO_2 after an equilibration period.

d. HPLC Distribution Pattern for SO_2 Data

The distribution pattern of BaP and metabolite for SO_2 data by HPLC are shown in Tables 47 and 48. There are no major changes except for the 7,8-dihydrodiol on tlc which has been broken down into the S metabolite and the 7,8-dihydrodiol on HPLC (Table 48). There are a few changes in the total dione and phenol percentage, but that is not completely unexpected. On the whole, the HPLC complements the tlc data but at the same time indicates the tlc is still a very valid method for analysis of BaP metabolites.

TABLE 47.

EFFECTS OF SO_2 AND PARTICULATE ON BaP METABOLISM*
% OF TOTAL BaP AND TOTAL METABOLITE REMAINING IN EACH TISSUE ± S.E.

Pretreatment	SO_2	-	-
IPL	BaP	SO_2 + BaP	SO_2+BaP+CAP
No. of Animals	3	3	2
% Unmetabolized BaP	44.4 ± 4.1	49.1 ± 0.6	18.4 ± 10.6
% of Total Compound as Metabolite in Tissue			
Blood	21.6 ± 5.1	22.4 ± 2.1	25.6 ± 11.1
TB	5.8 ± 1.3	2.1 ± 0.6	10.1 ± 3.5
MAC	0.7 ± 0.1	1.1 ± 0.2	7.4 ± 3.8
WO	2.3 ± 0.3	2.8 ± 1.1	4.1 ± 0.6
Lung	25.2 ± 5.7	22.5 ± 2.9	34.4 ± 0.2
% of Total Compound as BaP in Tissue			
Blood	10.7 ± 3.2	15.1 ± 6.0	0.8 ± 0.5
TB	2.4 ± 0.7	2.9 ± 0.5	1.3 ± 0.7
MAC	1.8 ± 0.4	3.4 ± 1.2	2.1 ± 0.9
WO	2.8 ± 0.2	3.2 ± 0.9	0.1 ± 0.1
Lung	26.6 ± 3.0	24.5 ± 2.2	14.1 ± 7.5

*HPLC data includes S value.

TABLE 48.

% DISTRIBUTION PATTERN OF BaP & METABOLITE IN EACH TISSUE

LUNG[x]

Pretreatment	SO_2	-	-
IPL	BaP	SO_2+BaP	SO_2+BaP+CAP
9,10-dihydrodiol	1.3±0.5	5.9±0.8	3.6±0.8
4,5-dihydrodiol	0.7±0.1	0.9±0.3	1.1±0.4
7,8-dihydrodiol	0.7±0.2	1.6±0.5	0.8±0.2
4,5-quinone	0.9±0.1	0.9±0.4	0.8±0.5
3,6-quinone	3.7±0.3	1.7±0.8	1.2±0.4
4,5-epoxide	1.0±0.3	1.3±0.4	0.7±0.4
9,6-OH	0.8±0.2	1.8±0.1	1.0±0.3
7-OH	0.4±0.1	1.2±0.2	1.3±0.7
3-OH	0.3±0.1	2.3±1.1	3.1±1.6
Metabolite S	2.4±0.4	3.3±1.2	6.3±5.2
BaP	77.3±2.2	69.0±1.8	70.2±12.0
Nonextractable	10.4±0.7	10.0±3.4	9.9±2.3

*120 min.

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,3,2 respectively - HPLC data.

(continued)

TABLE 48.

(continued)

180 BLOOD[x]

Pretreatment	SO_2*	-	-
IPL	BaP	SO_2+BaP	SO_2+BaP+CAP
9,10-dihydrodiol	2.1±0.8	10.1±2.6	11.3±6.9
4,5-dihydrodiol	0.9±0.3	1.7±0.5	4.0±1.3
7,8-dihydrodiol	1.1±0.1	2.3±0.6	4.1±1.7
4,5-quinone	1.0±0.1	1.6±0.5	3.2±1.8
3,6-quinone	3.2±0.2	2.1±0.7	3.2±0.8
4,5-epoxide	1.2±0.2	1.6±0.4	2.4±2.4
9,6-OH	1.0±0.2	0.9±0.5	2.9±1.4
7-OH	0.5±0.0	0.7±0.1	0.6±0.6
3-OH	0.4±0.2	0.7±0.5	3.4±0.0
Metabolite S	6.7±4.7	4.8±2.2	3.0±0.2
BaP	60.0±4.9	53.3±9.8	16.2±3.3
Nonextractable	23.6±3.2	20.2±9.8	45.4±6.6

*120 min.

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,3,2 respectively - HPLC data.

(continued)

TABLE 48.

(continued)

MACROPHAGE^x

Pretreatment	SO_2	-	-
IPL	BaP	SO_2+BaP	SO_2+BaP+CAP
9,10-dihydrodiol	0.7±0.2	2.1±0.1	1.1±0.2
4,5-dihydrodiol	0.5±0.1	0.9±0.5	1.4±1.0
7,8-dihydrodiol	0.5±0.0	0.8±0.3	1.0±0.6
4,5-quinone	0.7±0.1	1.2±0.8	0.6±0.3
3,6-quinone	1.2±0.2	1.0±0.7	2.1±1.9
4,5-epoxide	0.6±0.3	1.4±0.5	0.9±0.6
9,6-OH	0.6±0.0	1.1±0.3	1.5±0.7
7-OH	0.7±0.1	0.8±0.2	1.1±0.4
3-OH	0.9±0.4	1.3±0.5	1.0±0.1
Metabolite S	2.9±0.4	3.9±1.8	7.0±4.6
BaP	89.4±0.3	83.7±6.2	81.2±4.3
Nonextractable	1.5±0.2	1.9±0.7	1.1±0.2

*120 min.

^{x}% Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,3,2 respectively - HPLC data.

(continued)

TABLE 48.

(continued)

WASHOUT[x]

Pretreatment	SO_2	-	-
IPL	BaP	SO_2+BaP	SO_2+BaP+CAP
9,10-dihydrodiol	2.3±1.0	14.2±0.9	11.3±4.2
4,5-dihydrodiol	0.7±0.1	1.1±0.3	3.4±1.5
7,8-dihydrodiol	0.9±0.3	2.1±0.5	2.0±0.2
4,5-quinone	1.1±0.1	1.2±0.6	1.7±1.2
3,6-quinone	1.4±0.5	0.9±0.6	5.3±2.0
4,5-epoxide	0.7±0.2	0.9±0.2	2.8±2.3
9,6-OH	1.1±0.7	2.0±0.4	2.0±0.1
7-OH	1.2±0.4	1.0±0.3	1.6±0.7
3-OH	1.3±0.2	2.0±0.7	1.3±0.7
Metabolite S	3.2±0.9	6.4±3.6	5.4±0.2
BaP	78.8±3.7	60.7±8.3	17.3±7.6
Nonextractable	7.4±2.3	7.9±2.1	44.0±11.1

*120 min.

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,3,2 respectively - HPLC data.

(continued)

TABLE 48.

(continued)

TRACHEA BRONCHI[x]

Pretreatment	SO_2	-	-
IPL	BaP	SO_2+BaP	SO_2+BaP+CAP
9,10-dihydrodiol	1.9±0.6	3.4±0.9	3.2±0.2
4,5-dihydrodiol	0.9±0.3	0.9±0.1	1.7±0.7
7,8-dihydrodiol	1.5±0.6	0.9±0.1	2.7±1.5
4,5-quinone	1.3±0.2	0.5±0.0	1.5±0.4
3,6-quinone	2.2±0.5	0.8±0.2	3.0±2.5
4,5-epoxide	0.8±0.1	0.8±0.1	2.4±1.0
9,6-OH	0.7±0.1	0.6±0.1	0.4±0.4
7-OH	0.7±0.1	0.5±0.2	0.9±0.3
3-OH	0.4±0.1	0.7±0.2	3.0±0.5
Metabolite S	5.1±1.9	4.9±0.6	8.7±5.7
BaP	55.8±3.5	74.8±4.4	45.6±5.6
Nonextractable	22.8±6.6	11.3±3.4	26.9±5.4

*120 min.

[x] % Total activity at 180 minutes in each tissue ± S.E.

Number of animals are 3,3,2 respectively - HPLC data.

F. DISCUSSION

1. Perfusion - Basic Requirements

Investigations of benzo(a)pyrene metabolism have indicated that the metabolites are distributed differentially when comparing plasma to red cells (7). This observation reflects the importance of using whole blood when possible, since distribution, absorption, and excretion kinetics are important parameters in estimating total toxicity of a chemical. The possibility exists where significant factors may be overlooked when organs are perfused with artificial media. Another reason is that this perfusate is perhaps the best physiological and biochemical medium available, i.e., the essential cofactors, trace metals, and autologous protiens are present. However, the design of the experiment may dictate the use of artificial media, as for example in the study of lipid metabolism.

Constant blood pressure and flow are essential for kinetic studies. The system is essentially chemically and biochemically inert utilizing silicone rubber tubing and silicone coated glass. The system is operated at 37°C, and ventilation is accomplished through subatmospheric alternating pressures. The pH of the blood is controlled through infustion of NaHCO (0.3 meq/hr) and titrating to pH 7.40 with carbon dioxide added to the ventilating gas. The materials that are available for analysis include blood, lung washings, alveolar macrophages, trachea bronchi, peripheral lung tissue, and ventilating gases.

A summary of the biochemical changes found in the plasma of eight control isolated perfused rabbit lungs are found in Table 49. One of the most notable changes is the glucose concentration. The average disappearance rate was approximately 35 mg-%/hr. Infustion of this amount resulted in no net change throughout the perfusion. Lactate dehydrogenase, glutamic oxalacetic transaminase (GOT), and lactic acid were found to increase quite substantially. The increases in LDH and GOT and the increases in plasma hemoglobin were attributed to hemolysis (44).

Typical physiological values obtained from control lungs are shown in Table 50. Historically, edema has been a problem in the IPL. Minimization of edema has been evidenced by the small increases in weight of the lungs measured before and after perfusion.

Cervical dislocation was used to kill the animal since anesthesia was undesirable for metabolic studies; the location of the strike must be precise, since brain trauma results in massive hemorrhage and edema in the lungs. More recently, the lungs have been removed following CO_2 inhalation. We have also noted that in experiments in which the blood flow decreases with time in the IPL, edema and hemorrhage usually follows. Corrections were made on the blood flow problems that normally are found with the IPL hoping that these corrections would influence edema formation.

Figure 11 shows typical blood flow values which we considered inadequate for metabolic studies before the corrective measures were made. It was evident that in 1-2 hours a consistent phenomenon was occurring. Areas of hemorrhage and low perfusion were very evident in these lungs. Administration of regular maintenance doses of heparin (200-500 IU) as well as epinephrine (40-100 μg) were found necessary to maintain constant blood flow in the isolated perfused rabbit lung (Figure 12). Figure 12 illustrates the effects on blood flow of benzo(a)pyrene (BaP) in an ethanol saline (1:1, v/v) suspension administered intratracheally to the IPL. The initial decrease in flow is due to the ethanol administration. Histopathological examination of control lungs revealed no edema and excellent maintenance of pulmonary structures after 4 hr. of perfusion.

TABLE 49.

BIOCHEMICAL CHANGES IN THE PLASMA FROM BLOOD PERFUSING THE ISOLATED LUNG

	Mean Concentrations in Plasma Prior to Perfusion	Change in Mean Concentration Per Hour
calcium (mg %)	13.8±0.8	dec. 0.15±0.29
inorganic phosphate (mg %)	4.1±0.4	inc. 0.78±0.27
glucose (mg %)	236±35	dec. 34.5±4.1
adding 30 mg/hr		±2.3
blood urea nitrogen (mg %)	17.5±3.2	inc. 0.14±0.22
uric acid (mg %)	0.62±0.18	inc. 0.20±0.12
cholesterol (mg %)	37±13	inc. 4.2±0.7
with Vitamin E		inc. 19.5±7.1
total protein (gm %)	5.7±0.4	inc. 0.10±0.15
albumin (gm %)	0.53±0.07	dec. 0.04±0.03
total bilirubin (mg %)	0.14±0.06	inc. 0.12±0.16
alkaline phosphatase (mU/ml)	55±36	inc. 4.8±2.1
with Vitamin E		inc. 0.6±1.1
lactate dehydrogenase (mU/ml)	135±42	inc. 485±201
SGOT (mU/ml)	58±23	inc. 121±58
plasma hemoglobin (mg %)	0.19±0.12	inc. 2.3±0.6
lactic acid (mg %)	173±17	inc. 19.8±5.2
pyruvic acid (mg %)	1.19±0.06	N.C.* ±0.01

*No change

TABLE 50.

PHYSIOLOGICAL VALUES IN THE ISOLATED PERFUSED LUNG PREPARATION

hematocrit (%)	mean value = 35.0±5.0; mean change/hr. = dec. 1.6±0.3
weight gain (%/hr)	inc. 2.81±1.36
blood flow (ml/min)	approx. 160-210 (constant in each experiment)
P_{O_2} (mm Hg)	typical values 118±6; 121±10
P_{CO_2} (mm Hg)	typical values 39±4; 32±4
pH range	7.38 - 7.42
tidal volume (ml)	typical values 11.7±0.3; 11.0±0.4 published values 23.9±5.5 (Caldwell & Fry, 1969)

FIGURE 11

Inadequate Blood Flow Rate

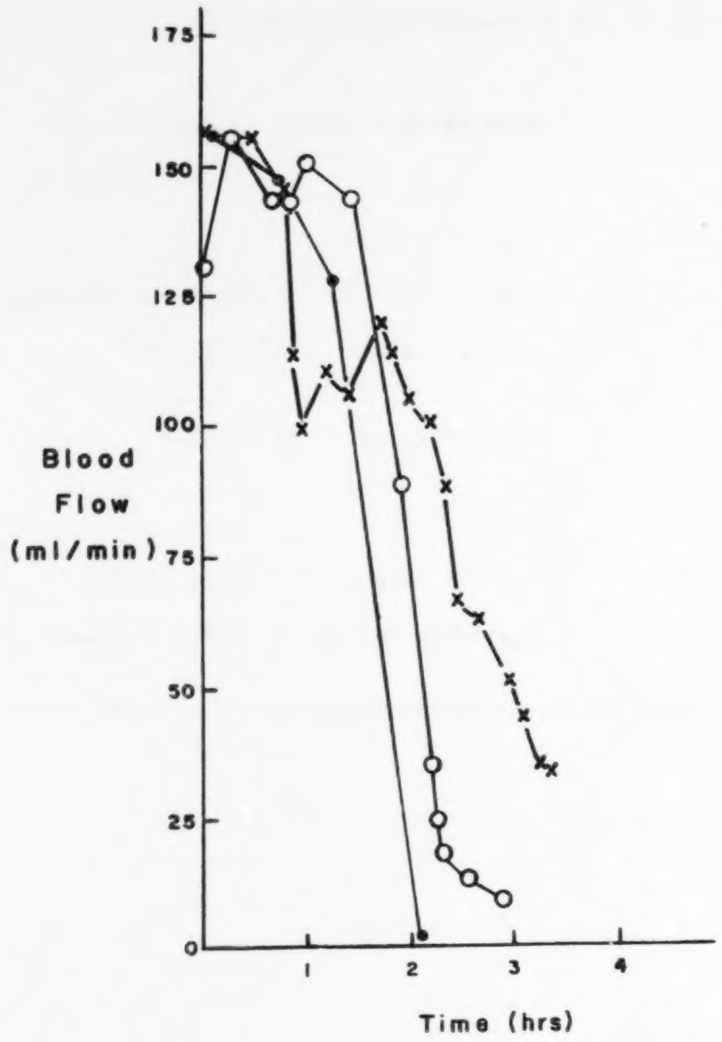
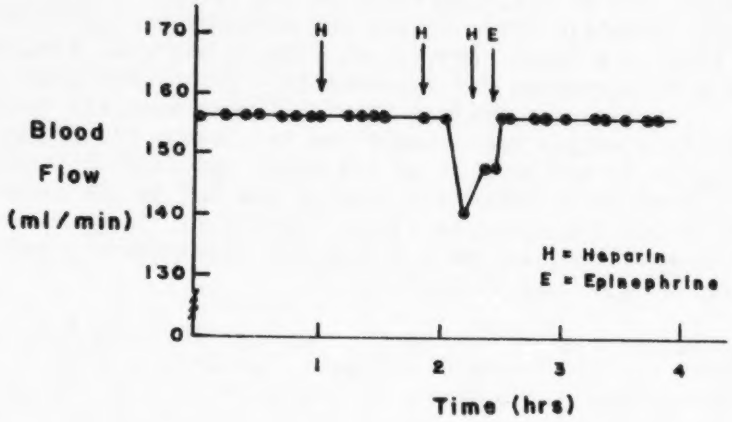
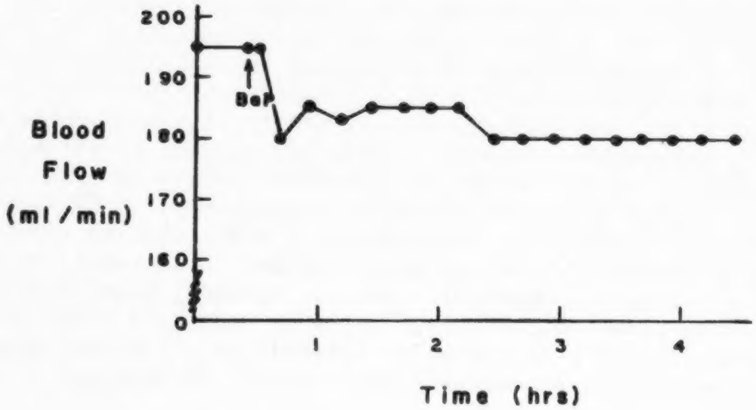


FIGURE 12



Blood Flow Rate After
Addition of Heparin and Epinephrine



Typical Blood Flow Rate
With BaP, Heparin and Epinephrine
Addition

2. Control Animals With BaP on IPL

Table 51 shows the distribution of radioactivity in the tissue of the isolated perfused lung preparation 1 and 3 hours after intratracheal administration of BaP. After 1 hour of perfusion, approximately 94% of the total activity remained in the lavaged lungs. At 3 hours of perfusion, the proportion of total activity in the lavaged lungs had decreased to approximately 60%. Plasma and erythrocytes combined contained only 4.5% of the total activity at 1 hour, while at 3 hours these same two components accounted for approximately 24% of the total activity. The red blood cells always contained about 50% more activity than the plasma. Pulmonary alveolar macrophages and the lavage fluid used in harvesting these cells had only 1% of the total radioactivity at 1 hour of perfusion. Three hours after addition of the BaP to the preparation, this distribution had increased to 15% of the total. Based on wet weight, the pulmonary alveolar macrophages had approximately twice the activity as the lavaged lung tissue.

The compounds recovered from these fractions included BaP, 3,6-quinone or dione; and its probable metabolic precursor, 3-hydroxy-BaP. In addition, three dihydrodihydroxys or dihydrodiols were tentatively identified on the chromatograms. The K-region 4,5-dihydrodiol was present. Two others were identified by their similarities to published R_1 values and qualitative fluorescent characteristics as the 7,8-dihydrodiol and the 9,10-dihydrodiol (tlc data).

A polar compound or compounds constituted approximately 40% of the total metabolites in the blood after 3 hours of perfusion. A time-course study using whole blood revealed that the extraction efficiency of the solvent was decreasing with time. Exhaustive solvent extraction with various types of solvent systems did not greatly increase the amount of radioactivity recoverable from the blood. Therefore, it was assumed that this radioactivity, which increased logarithmically with time, could be attributed to a very polar metabolite or metabolites. B-Glucuronidase did not affect its recovery. Digestion in 1 N HCl doubled the amount of extractable radioactivity and sulfatase incubation increased the extractable activity to comparable levels. However, substantial amounts of radioactivity remained. The radioactivity remaining after acetone: benzene extraction was with the polar metabolites. Further identification of this metabolite or metabolites is still in progress.

Table 52 shows the distribution of BaP and its metabolites in the tissue of the isolated perfused rabbit lung preparation 3 hours after the intratracheal addition of BaP. Control studies using circulating blood without the lungs yielded only the 1,6-dione, a chemical oxidation product of BaP, at only about 0.6%/hour. None of the other metabolites were encountered.

In the perfused lung preparation, very little parent compound (11021% of total) was found in plasma or erythrocytes; most was in the lungs and lavage fluid. However, whole blood did contain approximately 40% of the total metabolite produced by the lungs after 3 hours. About 45% of this metabolite remained in the lavaged lungs and the remaining 15% was found in the cell-free lavage fluid. This fluid had proportional amounts of individual metabolites, total metabolites, and BaP, which was very similar to those seen in lavaged lung tissue. Harvested alveolar macrophages contained only a small amount of metabolites and a much larger percentage of unmetabolized BaP.

3. Perturbations With IPL

A summary of the perturbations that have been completed or planned are presented in Table 53. This table reflects some of the potential uses of the IPL in characterizing the effects of many environmental contaminants of pulmonary metabolic activity. In addition, concurrent administration of multiple agents are made possible with this system for the purpose of investigating combined effects of agents in different physical forms.

TABLE 51.

DISTRIBUTION OF METABOLITES IN LUNG

Compound	Tissue				
	Plasma	Erythro-cytes	Alveolar Macrophage	Lavage Fluid (cell-free)	Lung
Total Metabolite[a]	89.1	78.9	10.6	42.1	39.3
Polar	51.2	46.3	3.2	21.2	18.9
7,8-dihydrodiol	6.5	2.4	1.2	2.9	6.2
9,10-dihydrodiol	25.9	14.6	1.4	8.4	6.4
4,5-dihydrodiol	1.7	4.3	0.7	2.3	0.5
3-hydroxy	2.7	8.0	2.2	7.3	7.3
3,6-dione	1.1	3.3	1.9		
B(a)P[a]	10.9	21.1	89.4	57.9	60.7
Total Metabolite, ug	16.7	27.1	1.2	15.9	48.3
B(a)P, ug	2.0	7.3	10.1	21.9	74.7

[a] Values represent percent of total activity in the tissue.

TABLE 52.

DISTRIBUTION OF RADIOACTIVITY

(% of total)

	1 hr.	3 hr.
plasma	1.86	9.10
RBC	2.87	14.90
lung	94.10	60.65
alveolar macrophage	0.89	5.57
lavage fluid (cell free)	0.28	9.78
TOTAL	100.00	100.00

TABLE 53.

PERTURBATIONS PRIOR TO PERFUSION

1. Enzyme Inducing Agents (ip)

   PB, 3-MC, B(a)P, PCB's

2. Inhalation Exposure

   SO_2, n-dodecane, coal dust, metals

3. Dietary Manipulations

4. Intratracheal Instillations

   B(a)P, crystalline quartz, papain, asbestos

   $\left.\begin{array}{l}\text{Ferric Oxide}\\ \text{Crude Air Particulate (CAP)}\end{array}\right] \pm \text{B(a)P}$

CONCURRENT ADMINISTRATION OF MULTIPLE AGENTS TO IPL

CAP + B(a)P $\pm$ SO_2

Ferric Oxide + B(a)P

Ethanol + Trichloroethylene

4. Effects of Enzyme Inducers

Phenobarbital does not induce the aryl hydrocarbon hydroxylase enzyme system (P_1450). Corn oil, on the other hand, is significantly different from the control which indicates that corn oil does induce the enzyme system. The metabolic profile for corn oil and the control are similar with less phenol and quinone and more 7,8-dihydrodiol formation for corn oil pretreatment.

Both BaP_{IP} and BaP_{IT} pretreatment cause increases in the rate of appearance of BaP metabolites in the blood. This is due to the fact that BaP will induce the P_1450 enzyme system (64). The metabolic profile shows a marked increase in the 9,10-dihydrodiol for BaP_{IP} or BaP_{IT} pretreatment while there is a significant decrease in the monohydroxylated and dione for BaP_{IT} from control and BaP_{IP} pretreatment.

There are also large changes in the distribution of BaP and its metabolites in the blood and lung. With concomitant increases in total metabolite in the blood and lung, there are corresponding decreases of BaP in these tissues. These major changes are consistent with increases in nonextractables in the blood and lung and with increases in the 9,10-dihydrodiol in the blood. This suggests that as more intermediate epoxides are formed at a faster rate, they are converted into conjugates of BaP or bound to macromolecules (64). Additionally, as the pathway becomes saturated the epoxide hydrase converts the 9,10-epoxide to 9,10-dihydrodiol and excretes it into the blood in both cases and rearrangements and/or isomerization converts some intermediates to phenols and quinones in BaP_{IP} pretreatment.

These data suggest that even though BaP is given by two different rates of administration, the rates of metabolism are significantly higher than the control in both cases and similar enzyme systems are induced. In one case, the enzyme levels are increased in whole animal but specifically in the liver 24 hours later, while in the second case the enzyme levels are increased in the lung specifically over a six week period.

3MC is not as good an enzyme inducer (P_1450) as BaP as indicated by the total rate of metabolism. The total rate can partially be accounted for by the corn oil administration. 3MC definitely causes an increase in the rate of metabolism compared to the corn oil and control, but is not significantly different at the 90 percent significance level (60). The metabolic pattern indicates that 3MC and BaP have similar profiles which are different from the corn oil control. 3MC and BaP appear to stimulate 9,10-dihydrodiol production.

These data for 3MC and BaP suggest that the metabolite patterns produced by P_1 450 enzyme inducers are similar and that more polar material is excreted into the blood stream than the appropriate controls. The turnover rate is faster and, therefore, the increased amount of epoxides that are formed as intermediates rearrange or isomerize to phenols and quinones or open up to 9,10-dihydrodiols by epoxide hydrase action.

This system, therefore, appears to be a good model system for stydying the metabolism of BaP. In recent studies (49,62) incubation of BaP in human and rat lung microsomes produced 30% and 20% total dihydrodiol respectively. This is consistent with our results for rabbit IPL in the production of 25-30% dihydrodiol formation depending on experimental conditions (68).

5. Particulate Effects

When particulate is administered with BaP on the IPL, the particulate inhibits the rate of appearance of metabolites of BaP observed in the blood. This can be explained by the relative increase of BaP and metabolite in the macrophage and washout with corresponding decrease of BaP in the lung and trachea bronchi. The BaP adsorbed particulate can be engulfed more readily by macrophages and, therefore, BaP is less biologically available for metabolism or is leached more slowly from the lung. However, at the end of 180 minutes the relative amount of BaP metabolized by CAP plus BaP is greater than the control which is consistent with an increase of metabolite in lung and trachea bronchi, as well as, washout and macrophage. This, then, might suggest that the CAP which contains a variety of PAHs and metals might induce the enzyme system slightly after a period of time for equilibration or that the macrophage metabolize BaP but delay the excretion of these metabolites in the blood.

When BaP is given IP to the whole animal, the rate of appearance of metabolites is increased significantly compared to its control. However, when CAP is introduced with BaP on the perfusion the rate of appearance of metabolites is decreased. The rate of appearance and the metabolite profile are similar to the corn oil pretreatment which indicates that CAP negates the effect of pretreatment. However, as the perfusion proceeds through 180 minutes, more BaP is metabolized, i.e. more BaP is available by leaching from CAP. In fact, the profiles for BaP_{IP} pretreatment followed by BaP alone and BaP plus CAP on the IPL are similar which suggests that after equilibration for a period of time, more of the BaP adsorbed on CAP is available for metabolism. This is reflected in larger amounts of nonextractables and 9,10-diol excreted into the blood with corresponding decrease of BaP. However, there are still minor differences at 180 minutes in that slightly more BaP and less metabolites are found in macrophage, lung, and trachea bronchi after introduction of CAP. With additional time, more BaP may become available for metabolism and, therefore, these differences could disappear.

CAP given as a pretreatment acts as an enzyme inducer to increase the rate of metabolism of BaP. BaP adsorbed on CAP administered as a pretreatment also acts like an enzyme inducer by increasing the rate of metabolism but the rate of metabolism is not the sum of the individual rates. This indicates that BaP is not leached readily from CAP, i.e. BaP is not available for enzyme induction. Overall CAP or BaP by itself increase the diol formation in the tissues with smaller increases or decreases in nonextractables. Together BaP and CAP decrease the diol formation and increase the nonextractables in the tissues.

When CAP is given as a pretreatment followed by BaP plus CAP on perfusion, the metabolism of BaP is increased compared to CAP pretreatment alone. Overall, these observations are reflected in decreases in diol formation and increases in BaP content. The major increase of BaP is in the macrophage which is consistent with decrease in metabolism; the BaP adsorbed or CAP administered to the IPL causes an increase in the rate of action of macrophage (5,19).

CAP appears to act as a cocarcinogenic agent with BaP. CAP acts as a physical agent in decreasing the biological availability of BaP or in the slower release of BaP over time when administered on the lung with BaP in comparison with pretreatment of particulate only. This data suggests that particulate affects the BaP metabolism by two different mechanisms: one mechanism appears to be a long-term effect due to pretreatment of particulate which causes an increase in the total metabolic activity and the other mechanism is a short-term effect due to particulate being administered to the IPL which causes a decrease in the total metabolic activity and inhibits the effects of pretreatment.

This work helps to partially clarify the ideas of a number of investigators (12,21,53,57,61). Particulates used in maintaining the environment of BaP in the lung for long periods appear responsible for increasing tumorigenic responses due to slow release of BaP from particulate. Also, particulates appear to influence metabolic pathways. The results presented indicate that both factors may be responsible, i.e. slower release of particulate as measured by appearance of metabolites in blood by IPL and a significant change in metabolic pathways.

With concomitant administration of particulate and BaP and, therefore, a slower release of rate, BaP is effectively administered to the lung tissue in small doses when compared to BaP itself. This type of treatment with particulate appears to be similar to previous observations: a carcinogen is much more effective in producing tumorigenic response when given in small divided doses over a period of time as opposed to single large equivalent doses.

6. SO_2 Effects

SO_2 pretreatment increases the rate of metabolism compared to control but does not significantly change the metabolite pattern. The SO pretreatment data, however, are very different from that obtained for an enzyme inducer which affects not only the rate but also the pattern. This difference in rate between an enzyme inducer, BaP, and SO_2 is reflected in a large increase in the 9,10-diol a slight decrease in the nonextractable.

After 180 minutes, similar results are obtained in that the amount of BaP unmetabolized in each case is consistent with rate of metabolism. In general, there are some small increases in the amount of nonextractables for SO_2 pretreatments compared to control while there are some rather large increases in the nonextractables for BaP_{IT} pretreatments compared to SO_2 and control. These data again suggest that SO_2 pretreatment show an increase in the rate of metabolism but does not significantly affect the pattern. SO_2, therefore, does not appear to affect BaP metabolism by enzymatic induction.

SO_2 administered on the IPL with BaP causes an increase in the rate of metabolism of BaP compared to control but does not significantly change the metabolic pattern. When CAP and SO_2 are administered concurrently with BaP on the IPL, the rate of metabolism of BaP is significantly decreased compared to SO_2 alone and does not change when compared to CAP alone. This could be due to the fact that BaP is not leached readily from CAP or to increased phagocytic agent. Additionally, at low concentrations of SO_2 the gas could very possibly be adsorbed by the particulate and is not available to affect the metabolism of BaP. The CAP and SO_2 together do cause small changes in the metabolic pattern compared to the control with increases in the 7,8- and 9,10-diol and a decrease in nonextractables.

At 180 minutes, the amount of unmetabolized BaP left for BaP plus SO_2 is consistent with rate of metabolism. SO_2, in vitro, does not appear to have major effects on the metabolite pattern. CAP plus SO_2 administered together with BaP, on the other hand, causes a significant increase in the rate of metabolism of BaP after an equilibration period. This can be due to the combination of leaching of BaP from CAP at a faster rate and changes in normal enzyme functions and/or cytopathological effects due to SO_2. Under these conditions SO_2 may cause an increase in enzyme activities.

Our attempt is to simulate environmental conditions. The 312 μg of BaP, 1 mg/kg of CAP, and 1-2 ppm of SO_2 used in the experiments are realistic human exposure values; however, there are some limitations in our system. The short time period will show only an immediate effect; with SO_2, CAP, and BaP together, it may be necessary to run the experiment for longer periods of time, perhaps at higher concentrations of SO_2, or to use larger amounts of BaP and CAP to obtain dose-response relationships, or to pretreat with SO_2 by inhalation (9). In the presence of CAP, the SO_2 can be adsorbed on particulates and some of the SO_2 may be catalytically converted to bisulfates under the right conditions of temperature and humidity: in the presence of sunlight, the SO_2 can be converted photochemically. We are more interested at this point, however, in the effects of SO_2 on BaP metabolism under our stricter environmental conditions (3,10,22,34,38,63).

SO_2 increases the metabolism of BaP by the IPL, but does not affect the metabolic pattern. It acts as a biochemical agent which causes biochemical changes in the lung due to irritation (10,42). More work in this area needs to be done in order to answer this question. The data indicate, however, that SO_2 can produce changes in the rate of metabolism of a well-defined carcinogen.

The presence of SO_2 at 1-2 ppm with CAP and BaP together indicates that SO_2 is either adsorbed by the particulate or that BaP is not available for metabolism due to either that BaP is not leached readily from the particulate, or an increase in phagocytic action of the macrophage which might serve to decrease the amount of BaP available for metabolism. The particulates, it should be mentioned, can maintain the environment of BaP in the lung for long periods of time which may be responsible for increased tumorigenic responses and altered metabolic pathways (12,20,53).

These studies are a first attempt to determine what type of interactions take place when SO_2 and/or CAP are added with BaP to a lung model system. This system appears to be a good model system for studying the metabolism of BaP in the presence of SO_2.

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